# ELECTRIC RESPONSES OF INDIVIDUAL NERVE ELEMENTS IN COCHLEAR NUCLEUS TO SOUND STIMULATION (GUINEA PIG)[1]

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## INTRODUCTION

IN A RECENT PAPER one of us (7) described the technique and the results of recording impulses from individual auditory nerve fibers in the modiolus of the guinea pig. The present investigation is an extension to the cochlear nucleus in the medulla oblongata of the same technique of recording action potentials with submicroscopic microelectrodes (6). Action potentials from single elements in the cochlear nucleus have already been successfully recorded by Galambos and Davis (2, 3, 4) with an earlier type of microelectrode. The original purpose of the present investigation was to repeat their previous observations with a finer technique.

There is a certain difference in behavior between the elements examined in the present investigation and those studied previously. The "response area" of an element is the area on an intensity-frequency plot that includes all tones that evoke a positive response. The response areas of the new elements show a sharp limit on the high-frequency side and very gradual elevation of threshold on the low-frequency side. The elements studied previously had, on the contrary, fairly narrow response areas with a clear maximum of sensitivity for a certain frequency that was characteristic of the element under observation. The new and the previous elements behaved differently in one other aspect: spontaneous discharges of impulses in the new elements were not inhibited by the application of any pure tone, while in the previous elements some tones inhibited and other tones enhanced the spontaneous repetitive activity.

## METHODS

Guinea pigs were anaesthetized with dial in urethane (approximately 0.05 cc./kg. body weight). A large incision was made through the skin over the masseter muscle, and the posterior half of the mandible was resected. Then a part of the sterno-cleido-mastoid muscle, the digastric muscle and the styloid process were removed to expose the postero-lateral part of the bulla. After opening the bulla a relatively large hole was drilled with a dental burr no. 1/2 through the thick bony wall of the cerebral cavity, starting between the posterior semicircular canal and the scala tympani of the cochlea and aiming toward the internal auditory meatus (Fig. 1). This technique is similar to that used in a previous

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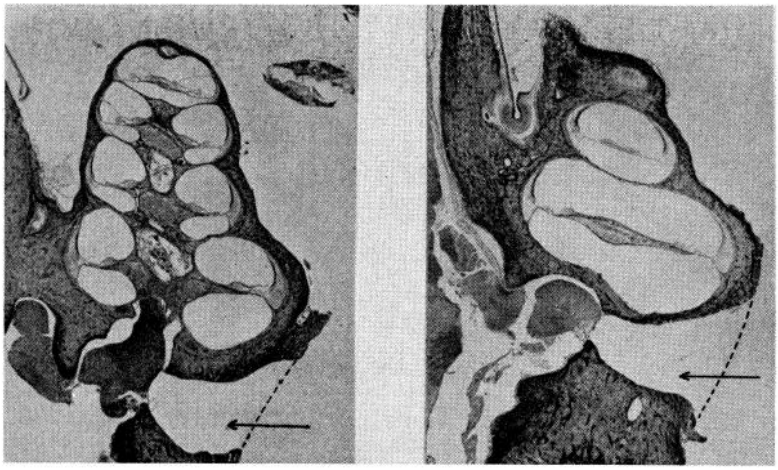

FIG. 1. These sections of guinea pig cochleas show typical holes, drilled inward from bulla, through which microelectrodes were introduced into medulla. In left (mid-modiolar) section note division between lighter cell-rich area of brain and darker auditory nerve through which electrode was inserted. In off-center section on right, gray matter of medulla is directly exposed. Dotted lines show approximately the original contours of bone. Arrows indicate holes that were drilled to admit microelectrodes.

experiment (7) except that the present holes were directed toward the origin of the eighth nerve in the medulla. The dura covering the medulla was removed with a small pair of watchmaker's forceps. A small piece of cotton wool was inserted in the hole to absorb the cerebro-spinal fluid that slowly accumulated, but immediately before introduction of a microelectrode the cotton wool was removed.

Microelectrodes were drawn by hand from glass tubes of about 1 mm. diameter. The tips of these pipettes were examined under a high-power optical microscope to reject those which did not taper off uniformly until the tip became invisible. A three-molar KCl solution was introduced into the pipettes by boiling them in pure ethanol and then replacing the ethanol with the concentrated KCl solution. The head of the animal was fixed rigidly to the table, and the microelectrode was slowly pushed into the eighth nerve at its origin in the medulla. In piercing the pia care was taken to avoid visible blood vessels. The KCl solution in the microelectrode was connected to the grid of a cathode-follower with a short piece of fine silver wire. To record the microphonic and whole-nerve responses to sound stimuli, a nichrome-steel wire electrode was inserted into the scala vestibuli of the basal turn of the cochlea. The potentials of the microelectrode and of the metal wire electrode, both referred to an indifferent electrode placed on the neck of the animal, were recorded simultaneously through two cathode-follower preamplifiers and condenser-coupled amplifiers. DC potentials at the tip of the microelectrode were also often observed on the screen of the oscillograph but were not photographed.

Sound stimuli were either tone pips (1) or pure tones applied through the opening in the bulla. The external auditory meatus of the animal was roughly closed with a piece of plasticene and a clip. The transducer used was a Lansing speaker (D-175) placed at a distance of approximately 0.5 m. from the animal and directed toward the animal's bulla.

## EXPERIMENTAL PROCEDURE AND RESULTS

The procedure of the experiment was in general as follows: 500 cps tone-pips of a moderate intensity were delivered at a rate of 6–10 pips per sec. These evoked microphonic responses of 150–300 $\mu$V. peak-to-peak in scala vestibuli of the basal turn (8). The microelectrode was slowly pushed into

the medulla. When the microelectrode began to pick up distinct unitary responses, the filter circuit for generating tone-pips was altered to give pips of 8000 cps, and its intensity was adjusted for maximal nerve response ($N_1$). A series of responses to tone-pips was photographed and then, if the unitary response still remained unchanged, pure tones were substituted as stimuli.

In successfully operated animals in which 8000 cps tone-pips elicited whole-nerve responses of greater than 100 $\mu$V. (maximal) after the end of the

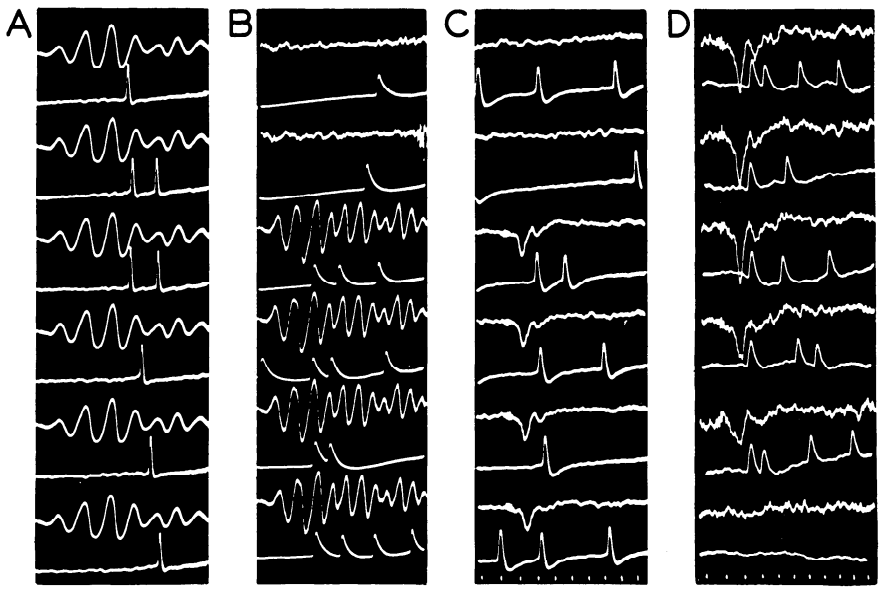


FIG. 2. Four series of responses to tone-pips photographed on continuously moving film. Upper beam of twin-beam oscilloscope records cochlea microphonic and also action potentials of the whole nerve (from scala vestibuli of basal turn referred to neck). Lower beam records unitary responses picked up by microelectrode in cochlear nucleus. Upward excursion indicates increasing positivity of search electrode. Stimuli for A and B are 500 cps tone-pips, for C and D 8000 cps tone-pips. Responses in upper tracings of A and B are chiefly cochlear microphonics, in C and D chiefly action potentials, "$N_1$" and "$N_2$". Time marker at bottom of C and D shows msec. Original voltage calibrations were: upper beam in A and C 110 $\mu$V./cm., in B and D 34 $\mu$V./cm.; lower beam (unitary responses) in A, B and C 1.1 mV./cm., in D 0.24 mV./cm. Strength of stimulus adjusted in C and D to give maximal $N_1$ response in upper beam.

operation, it was very easy to obtain distinct unitary responses to sound stimuli with a microelectrode pushed into the region of the cochlear nucleus. In several favorable cases we recorded from as many as 15 different units in the same preparation. All of these single-unit responses were positive spikes less than 1 msec. in duration. The shape of the spike was sometimes monophasic and triangular (Fig. 2B, D) while in other cases it was slightly diphasic and shorter (Fig. 2A, C). The spike height of our single-element responses was in general smaller than 10 mV. We recorded action potentials from more than 80 different units in the region of the cochlear nucleus, but

in none of them was the main deflection negative at the tip of the exploring microelectrode.

Many of the units which responded to 500 cps pips showed definite responses to 8000 cps tone-pips also. The latency of the unitary responses to this high-frequency tone-pip was in many cases definitely longer than that previously observed by one of us (I.T.) with the exploring electrode inserted into the auditory nerve in the modiolus (7). When the position of the hole was as shown by the left photograph in Fig. 1, the microelectrode at superficial levels presumably picked up responses of the auditory nerve fibers, and

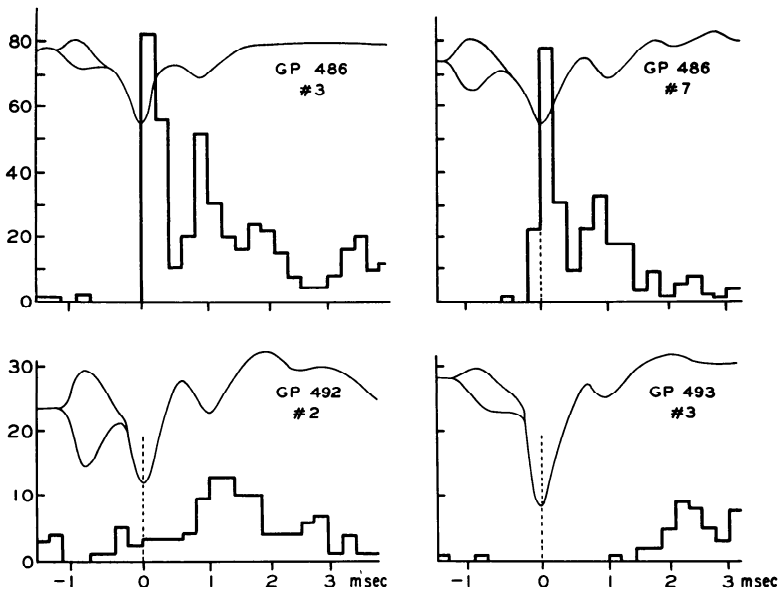


FIG. 3. Distribution of latencies of unitary responses from cochlear nucleus. Ordinate, number of responses; abscissa, latency in msec. measured from peak of $N_1$ (recorded simultaneously from basal turn) to foot of each unitary response. The few responses that preceded $N_1$ are spontaneous discharges. Form of a typical cochlear response is indicated above each histogram. Double line preceding first downward peak ($N_1$) in this tracing shows envelope of cochlear microphonic. Second negative peak at 1 msec. is $N_2$. Stimuli, 8000 cps tone-pips adjusted to give a maximal $N_1$.

as the microelectrode was advanced into deeper positions it presumably recorded responses of the secondary neurons in the auditory system. In fact, many of the units responded, as the auditory fibers did (7), synchronously with the peak of the whole-nerve response. In some other units the response appeared slightly after the negative peak, and in still others near or even after the so-called $N_2$-peak which follows the first large negative peak at an interval of approximately 1 msec.

In Fig. 3 are shown the latencies of the spikes recorded from four different units in the cochlear nucleus which responded to 8000 cps tone-pips. In this figure the number of spikes observed was plotted as ordinate against

their latency as abscissa. In the two upper examples in this figure the first spike usually appeared approximately at the peak ($N_1$) of the whole-nerve response. In these two cases the second spike tended to appear approximately 1 msec. after the first spike. This is the interval between $N_1$ and $N_2$ responses. It is likely that our electrode was recording the responses of the primary neurons in these cases (cf. 7). It is significant, however, that some of the short-latency responses were recorded when the tip of the recording microelectrode was actually in the medulla and not in the auditory nerve.

The two lower examples in Fig. 3 show elements which responded at longer latencies. In these cases the variation in latency was greater than in the upper cases, and there are relatively few responses that correspond to the $N_1$ and $N_2$ responses in the whole nerve. They are probably from secondary or higher order neurons.

It was our impression that, with our present type of microelectrodes,

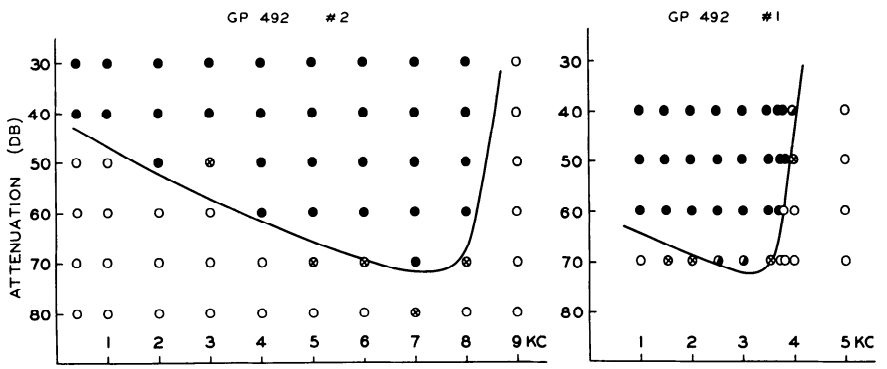


FIG. 4. Response areas of two elements in cochlear nucleus. Solid dots = clear strong response; half filled circle = weak, but recognizable; cross in circle = doubtful; empty circle = none. Abscissa is frequency of pure-tone stimulus in kilocycles. Ordinate is attenuation in decibels from an arbitrary high level. Responses of Element no. 1 (right) to tone-pips are shown in Fig. 2B.

single units in the region of the cochlear nucleus survived longer than the auditory nerve fibers in the modiolus. Most of the unitary responses in the present investigation remained unchanged for several minutes. On some exceptional occasions responses of a unit in the medulla stayed unchanged for more than 30 minutes. In the previous experiments on the auditory nerve fibers only a very few fibers responded for more than 2 or 3 minutes.

In Fig. 4 are shown data from two different units which remained active for more than 30 minutes. We could map out the response areas of these two units (from the same animal) with fair constancy. For this mapping the stimuli were brief bursts of pure tone. The on-effects were disregarded and the experimenter centered his attention on the rate of discharge of impulses heard in the monitor loudspeaker just after the first 0.5 sec. of stimulation. The response was considered positive if this rate was clearly faster than the

rate of spontaneous discharges between stimuli. Element no. 1 in this animal responded to 500 cps tone-pips (as shown in Fig. 2B) but it did not give any responses synchronized with the 8000 cps tone-pips. Its response area showed a very sharp cut-off just below 4000 cps and a gradual rise in threshold toward the low-frequency side. Element no. 2 showed good responses to both 500 cps and 8000 cps tone-pips. This element had its cut-off frequency at about 8500 cps. As can be seen in the figure, the shape of the response area is essentially similar to that of the primary neurons studied previously (7) but less sharp than the Galambos-Davis response areas (3).

On a few occasions the rate of discharge was examined as a function of stimulus frequency with the stimulus intensity held approximately constant (Fig. 5). Each tone was presented to the animal for approximately 2 sec. and the rate of steady impulse-discharge was determined by measuring photographic records of the responses after the first 0.5 sec. after the onset of the tone. The level of spontaneous discharge was very high in this preparation (as in Fig. 2C), but a gradual increase in the rate of discharge with increasing stimulus frequency and a sudden decrease in its rate at the cut-off frequency just below 4000 cps was clearly seen. Indeed, the continuous line in Fig. 5 looks very much like a mirror image of the curve of Fig. 4, right, which was actually taken from the same unit. Both curves express the effectiveness of sounds of different frequency in exciting a particular auditory unit.

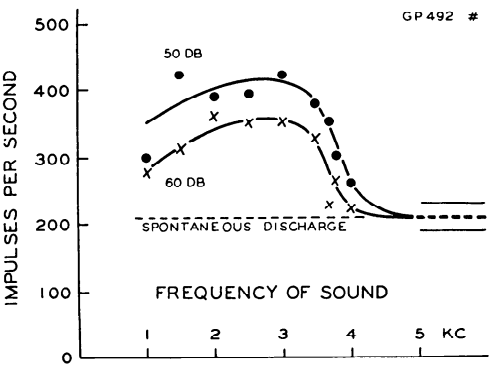


FIG. 5. Rate of discharge in a particular element as function of frequency and intensity of pure-tone stimulation. This element showed an unusually high rate of spontaneous discharge, 210/sec., that varied only over range shown by two horizontal lines at right. Each impulse count represented by dot or cross was made over an interval from 0.5 to 2.0 sec. after onset of a pure tone. Reference level for intensity is arbitrary. The two levels employed were subjectively of "moderate intensity."

In the region of the cochlear nucleus we found many elements which showed a fairly high rate of spontaneous discharge. In the elements shown in Fig. 2B (also Fig. 4, right) and Fig. 2C, for example, the average rate of discharge in the absence of stimulating sound was as high as 200 impulses per sec. We believe that this high rate is not due to injury of the element by the microelectrode because (i) the responses shown in Fig. 2C are strongly diphasic, (ii) the intervals between the individual impulses are irregular, (iii) in several cases in which the DC was measured there was apparently no large resting (intracellular) potential at the tip of the electrode, and (iv) no evidence of deterioration appeared in the form of a slowing of the discharge over periods up to 30 minutes or more. But in no element examined did sound stimuli ever stop or depress these spontaneous impulse discharges.

For example, in Element no. 1 of Fig. 5 the effect of strong high-frequency tones (at 30 db attenuation) was carefully examined between 5 and 10 kc., but no observable effect was produced by any tone above the cut-off frequency of this element.

## Discussion

The chief differences between the results of the present experiments on guinea pigs and the previous experiments by Galambos and Davis on cats are in the shape of the response area of the unit and the behavior of the spontaneous discharge. There are two probable explanations of these differences. One is simply the difference between guinea pigs and cats. The other—and more likely—explanation is that they are due to the difference in the size of the microelectrodes employed.

The insertion of a submicroscopic glass pipette into the *cell body* of a neuron injures the neuron seriously (9). The elements in both the present experiments and in the Galambos and Davis series responded regularly over considerable periods of time. It is therefore unlikely that the electrodes were actually inside the cell bodies. But when action potentials of cell bodies are recorded from *extra*-cellular placements of electrodes the responses are negative or diphasic. We conclude that the purely positive action potentials that we have recorded are actually the responses of *axons*, not of cell bodies. The responses of short latency are, we believe, the responses of primary neurons and the others with longer latency the responses of the secondary neurons.

The elements studied by Galambos and Davis (2, 3, 4) were undoubtedly cell bodies. Their recording electrodes detected strong potential fields and the responses did not deteriorate. This combination implies that their electrodes lay near but outside the cell bodies of cells with well-developed dendrites (cf. 9). The dendrites of the secondary neurons of the ventral cochlear nucleus are described by Cajal as "small" (cf. 5) but no such statement is made concerning the dorsal cochlear nucleus. It seems therefore possible that the elements of Galambos and Davis are some of the second-order neurons of the dorsal cochlear nucleus. It is unfortunate that no latency measurements were made to confirm either the present or earlier inferences as to the order of the neuron (cf. 4).

## Summary

Unitary electrical responses have been recorded from more than 80 elements in the cochlear nucleus of the guinea pig by means of submicroscopic micropipette electrodes. Their electrical polarity was always positive.

Spontaneously discharging elements, some with rates as high as 200/sec., were often encountered. The rate of spontaneous discharge could be increased by acoustic stimulation of proper intensity and frequency, but the discharge was never inhibited.

Individual impulses could often be correlated clearly with brief acoustic

stimuli (tone-pips). By the latency, wave form and electrical polarity of the responses some of the elements were identified as axons of primary neurons. Others seemed to be axons of secondary neurons.

When stimulated by brief bursts of pure tones the responding elements showed sharp frequency cut-offs at the upper boundaries of their response areas. Both first-order and second-order elements showed very flat maxima and a gradual fall of sensitivity on the low-frequency side.

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