

# UC Irvine

## UC Irvine Previously Published Works

### Title

Intracellular recordings from cat cochlear nucleus during tone stimulation.

### Permalink

<https://escholarship.org/uc/item/22t8w0qg>

### Journal

Journal of Neurophysiology, 33(1)

### ISSN

0022-3077

### Authors

Starr, A

Britt, R

### Publication Date

1970

### DOI

10.1152/jn.1970.33.1.137

### Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

# Intracellular Recordings from Cat Cochlear Nucleus During Tone Stimulation

ARNOLD STARR AND RICHARD BRITT

*Division of Neurology, Stanford University Medical Center, Stanford, California 94305*

ALL OF THE VIIIth NERVE FIBERS originating in the cochlea terminate in the cochlear nucleus, the first central station of the auditory pathway. Extracellular recordings from units in this nucleus have shown a diversity of both spontaneous and tone-evoked activities compared to activity in VIIIth nerve fibers. Tone-evoked inhibition of spontaneous activity occurs with certainty in the cochlear nucleus (8, 9, 12), but has not been securely demonstrated in VIIIth nerve (5, 9, 10, 18, 22), and response patterns to steady tones are quite varied in cochlear nucleus (15), whereas a single response pattern characterizes VIIIth nerve fibers (10). The synaptic events in cochlear nucleus that accompany acoustic stimulation have only recently begun to be explored (4, 6). The present study utilizes intracellular recordings and stimulating techniques in the cochlear nucleus to further delineate the synaptic basis for central processing of auditory signals.

## MATERIALS AND METHODS

Thirteen cats were studied. They were anesthetized with sodium pentobarbital (35 mg/kg initially and 30 mg every 3 hr thereafter), intubated, paralyzed with gallamine triethiodide (Flaxedil, 1 ml/hr), and artificially respired. Body temperature was maintained between 37 and 39 C. The animal was held by a face clamp, leaving the pinna and external auditory canals exposed. The lateral portion of the cerebellum was aspirated to expose the right dorsal cochlear nucleus, and a recording micropipette lowered to the surface of the nucleus. Brain pulsations were reduced by an agar solution placed over the exposed portions of the brain stem and by a pneumothorax. The animal was then placed in a sound-attenuating room separated from the experimenters and the recording equipment.

Received for publication August 4, 1969.

## *Sound signals*

Sinusoidal voltages for tonal signals were generated by a voltage-controlled oscillator, passed through an electronic switch for regulation of rise and decay times (5 msec), attenuated, and presented by an Altec 802 D speaker placed 15 cm in front of the right pinna. A staircase integrator that generated 42 equal voltage levels over a 20-sec period provided the voltages for regulating the frequency of the oscillator. The resulting acoustic signals were a series of ascending frequency tone bursts ( $f_1, f_2, f_3 \dots f_{42}$ ) with a constant number of Hertz separating adjacent tones in the sequence. The electronic switch gated each tone burst to be approximately 150–200 msec in duration. The output of this system measured by a  $\frac{1}{2}$  inch Bruel and Kjaer microphone at the level of the external auditory canal was  $\pm 5$  db from 1 to 14 kHz and  $\pm 9$  db from .5 to 22.0 kHz. Intensities up to 90 db SPL were used.

## *Data acquisition*

The micropipettes were filled with 2 M potassium citrate and had impedances between 10 and 35 megohms. A negative-capacitance circuit (bandpass dc-30 kHz) amplified the potential difference between the micropipette and an indifferent Ag-AgCl electrode placed on the tongue. A modified bridge circuit (2) was used, in the last seven animals, to pass current through the micropipette. The amplified activity was displayed on an oscilloscope and recorded on magnetic tape along with the oscillator control voltages, oscillator output, current pulses, and event markers for subsequent data processing.

## *Experimental procedures*

The micropipette was slowly advanced by remote control during tone presentation. Extracellular recordings of single units were characterized by biphasic spikes and little or no change in the d-c level. The electrode was considered intracellular or quasi-intracellular

(11) if there was a drop in the d-c level and cell discharges were of a positive polarity. The experimental protocol consisted of 1) presenting the sequence of ascending frequency tone bursts at several different intensity levels, beginning at 90 db SPL and then decreasing in 10 db steps; 2) presenting 40 trials of just one particular frequency at different intensity levels; and 3) passing intracellular currents during tone stimulation. Most of the units were lost before the entire protocol was completed.

### Data analysis

Resting membrane level, spike heights, and effects of sound stimulation were determined from kymographic records made of every unit recorded on magnetic tape. The tapes were also analyzed by a digital computer (LINC) as follows.

**RESPONSE-AREA HISTOGRAM.** The number of discharges occurring during each frequency tone burst for the different intensities tested.

**POSTSTIMULUS TIME HISTOGRAM (PSTH).** The number of discharges occurring during sequential 2-msec epochs of a tone burst. The analysis was initiated 10 msec before tone onset and included 40 trials of one tonal frequency.

**AVERAGE MEMBRANE POTENTIAL.** The shape of the membrane potential during tone stimulation for subsequent comparison with the shape of the PSTH. The analysis was initiated 10 msec before tone onset and included 40 trials of one tonal frequency; the digitizing rate was 2 msec. A low-pass filter (3 db down at 37.5 Hz, 20 db/octave slope) was used to attenuate spike discharges in the computation.

**INTEGRATED MEMBRANE LEVEL.** A sum of the difference between the membrane potential 10 msec before tone onset and the membrane potential every 10 msec during each frequency tone burst for the different intensities tested. The low-pass filter in (3) was used to attenuate spike discharges in the computation. The integrated membrane level is a measure of membrane potential change as a function of tonal frequency and intensity and can be compared with the response-area histogram.

### RESULTS

Intracellular and quasi-intracellular records were obtained from 58 cells in the cochlear nucleus. The electrode was judged intracellular if 1) a large negative d-c shift (20–70 mv) developed abruptly on encountering the cell, 2) the spike discharges were

of a positive polarity, and 3) both resting membrane level and discharges disappeared abruptly with movement of the electrode or in the course of study. Satisfactory intracellular recordings were maintained in 20 cells for periods ranging from 20 sec to 10 min. Data derived from the other 38 cells seemed compatible with an electrode in close proximity to the cell, but not yet intracellular, a recording situation termed quasi-intracellular by McIlwain and Creutzfeldt (11). Membrane potentials and spike amplitudes were of low amplitude when cells were first encountered and both would increase gradually on advancing the electrode. Eventually, injury discharges and loss of membrane potential accompanied one of the electrode movements. Our experimental procedure was to stop the electrode and to study responses to tone stimuli when the membrane potential was steady, the synaptic potentials clear, and the spikes of positive polarity. Recordings of up to 40 min were achieved with this technique from cells that had membrane levels between -10 and -40 mv and spike amplitudes of 1–15 mv.

The potential changes that accompanied tonal stimulation are illustrated in the intracellular records from one of our better units (Fig. 1). A sustained depolarization accompanied tones that evoked an increase

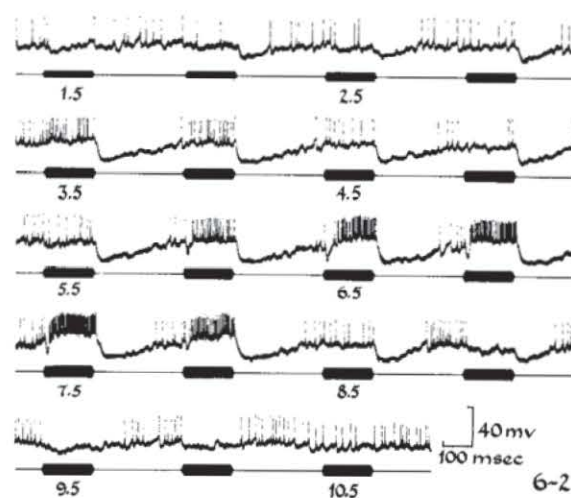


FIG. 1. Intracellular record (*upper trace*) from cell 6-2 during tones (*lower trace*) at 90 db SPL. Frequency of the tone is indicated on alternate tone bursts. Note depolarizing shifts with tones that increase discharge rate, and hyperpolarizing shifts with tones that suppress activity. A large hyperpolarization occurs at offset of most of the tones.

in discharge rate. Hyperpolarization during excitatory tone stimulation was occasionally noted when the electrode was in an extracellular location. On advancing the electrode to penetrate the cell, the hyperpolarizing response, which was never greater than 1 mv in amplitude, inverted to a depolarization of considerably larger magnitude (Fig. 2; see also Fig. 7 from ref. 9). Of the 49 cells excited by tonal stimulation 43 had an associated depolarization shift of up to 10 mv in amplitude, whereas no change in membrane potential could be detected in 6 cells. The particular configuration of the depolarizing shift varied from cell to cell as

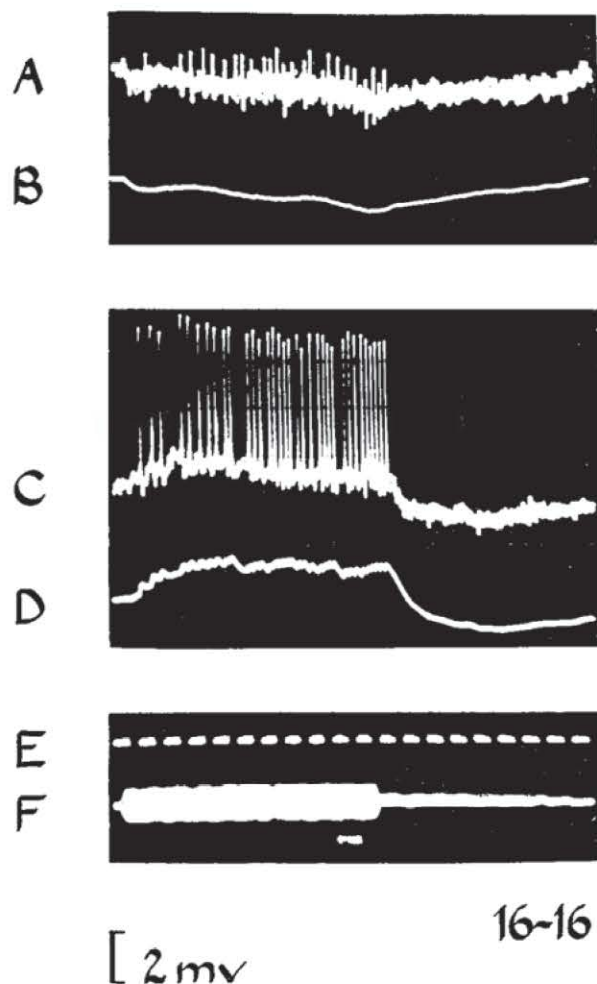


FIG. 2. Comparison of extracellular (*A* and *B*) and intracellular (*C* and *D*) records from cell 16-16 during stimulation at 7.5 kHz (*F*). A low-pass filter was used to attenuate spike discharges in traces *B* and *D*. Note biphasic spikes and sustained hyperpolarization in extracellular records. There was a transition of the spikes to a positive polarity and the appearance of sustained depolarization when the electrode penetrated the cell. Time scale (*E*) is 60/sec.

well as with the frequency and intensity of the tonal stimuli. In Fig. 1, for example, depolarizing shift was interrupted shortly after tone onset by a brief hyperpolarization during some frequencies (6.0–8.0 kHz) but not others (3.5–4.5 kHz). The relationship of the depolarizing shifts to the pattern of cell firings will be discussed in detail in a later section of the paper.

A sustained hyperpolarization was noted during stimulation with tones that suppressed spontaneous activity (Fig. 1, 1.5 kHz, 9.0–10.0 kHz), but suppression of spontaneous activity could also occur independent of any membrane potential change (see Fig. 5). Of the 29 cells showing a suppression of spontaneous activity with tone stimulation, 16 had sustained hyperpolarization shifts of up to 15 mv in amplitude whereas no change in membrane level could be detected in 13 cells.

Three types of membrane potential changes were noted on terminating the tone bursts. In 33 cells there was a profound hyperpolarization (up to 20 mv) that was maximal at tone offset and then gradually decreased in amplitude over the subsequent 200 msec. Spontaneous activity was suppressed throughout this period. Hyperpolarization at tone offset was prominent following excitatory tones but, as is evident in Fig. 1, also appeared following tones that either had no effect on spontaneous activity (3.0 and 8.5 kHz) or even suppressed spontaneous firings (9.0 kHz). In 8 cells, the hyperpolarization was interrupted 20–50 msec after tone offset by an abrupt depolarization that then persisted for 100–200 msec (see Fig. 5, 13 kHz, 90 db). This rebound depolarization was restricted to tones in the region of the “best” frequency if their intensity were at least 60 db above threshold. Finally, in 5 cells, stimulation with intense excitatory tone bursts (> 70 db SPL) resulted in a depolarization shift that persisted for 20–100 msec beyond the tone’s termination (Fig. 7, cell 13-1). The depolarizing shift, in 4 of these cells, was followed by the usual off-hyperpolarization.

The amplitudes of the depolarizing and hyperpolarizing shifts during tones and the hyperpolarization at tone offset varied from 1 to 20 mv and are plotted in Fig. 3

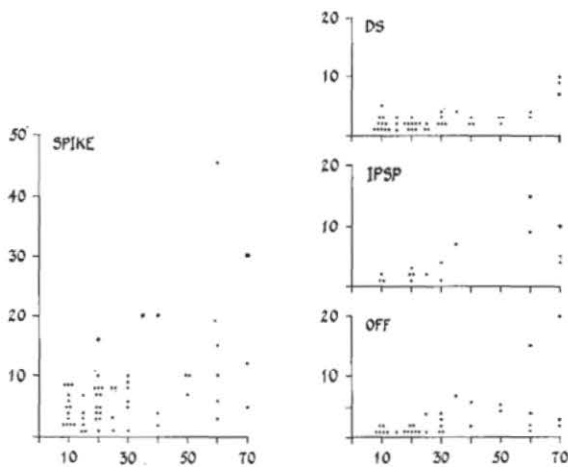


FIG. 3. Amplitudes of spike discharges (SPIKE), depolarizing shifts (DS), hyperpolarizing shifts (IPSP), and hyperpolarization at tone offset (OFF) as a function of resting membrane level (*abscissa*) for all cells studied in the present experiment. Scales are in millivolts.

as a function of the unit's resting membrane level. A plot of spike amplitude as a function of membrane level is also included in this figure. All of these measures tended to be larger in cells with greater resting membrane potentials.

#### *Relation between membrane potential changes and spike discharges*

In 12 units the number of discharges evoked by the different frequency tone

bursts presented over at least a 60-db intensity range (response-area histogram) were compared with the integrated membrane level evoked by these same signals. The measures were displayed as two tuning curves; one based on spike counts and the other on membrane potential change. Results from one of the intracellularly recorded units are graphed in Fig. 4 and show the two tuning curves to be in general accord except for the following: First, the range of tonal frequencies that excited the cell was more extensive when defined by membrane depolarization than by spike count. Second, tonal frequencies adjacent to the excitatory area could be classified as inhibitory by membrane hyperpolarization, whereas the absence of spontaneous discharges made it impossible to classify any frequency as inhibitory in spike-count measurements. Third, threshold as defined by membrane depolarization was at least 10 db lower at every frequency than threshold defined by the occurrence of cell discharges. Thus, for this cell, membrane potential measurements seem an accurate reflection of the synaptic events governing cell discharges. However, in 8 of the other 11 cells examined by this method, membrane potential changes were often at variance with the spike-count data. The results

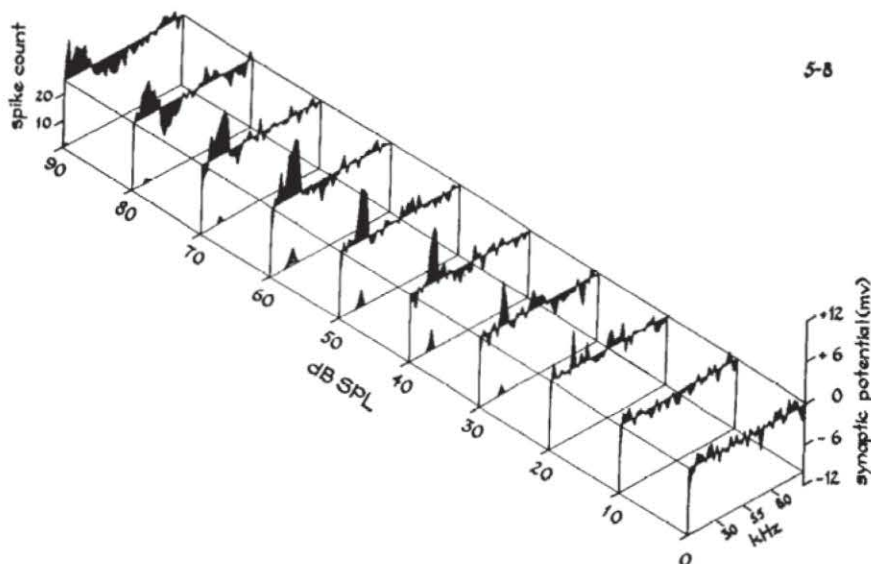


FIG. 4. Three-dimensional display of response-area histogram (lower level of figure) and integrated membrane level (upper level of figure) for cell 5-8 as a function of tone frequency and intensity. Scale of spike discharges for the response-area histogram is on the left side of the figure and the scale for membrane level changes is on the right side of the figure.

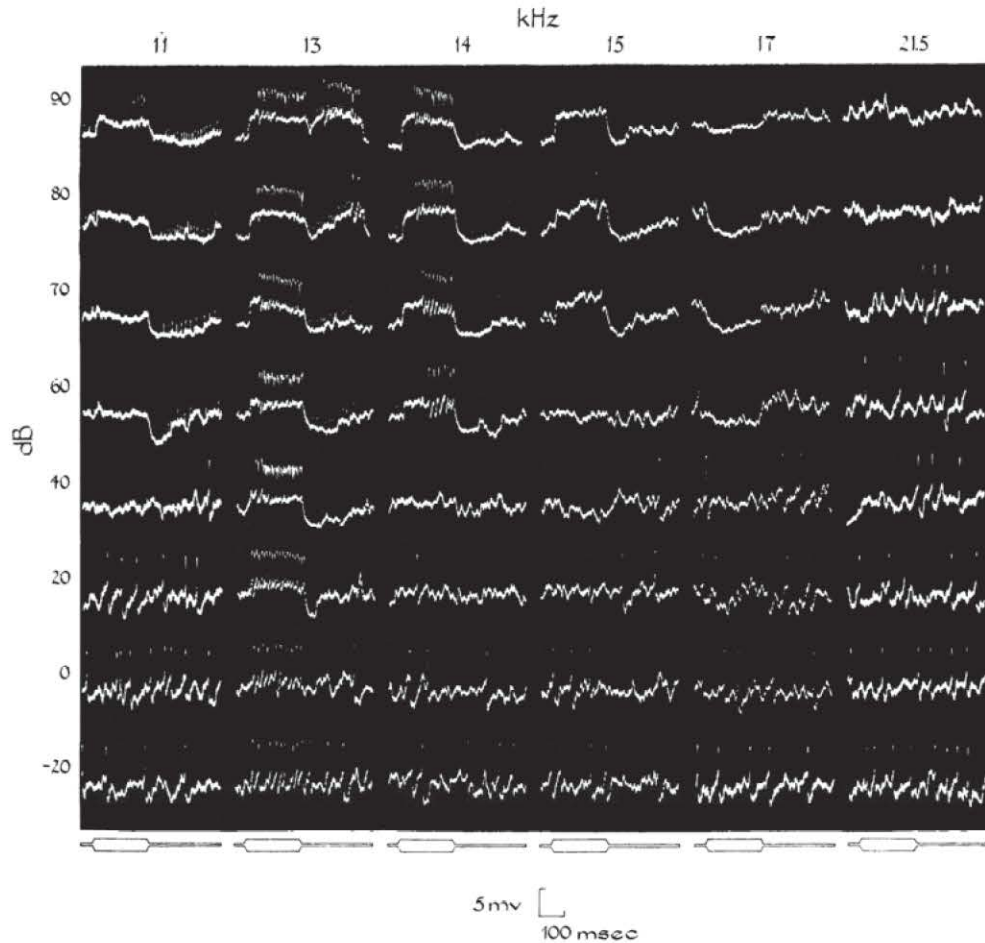


FIG. 5. Individual traces from cell 20-6 during six different tone signals over a 110-dB intensity range. Period of the tones is indicated at bottom of the figure. Dotted line in all traces represents firing level of the cell in the absence of tones. Note: 1) the variability of firing level and 2) the suppression of spontaneous discharges by tones with and without an accompanying hyperpolarizing shift.

from one of these cells (cell 20-6, Fig. 5) demonstrate the types of noncorrespondence noted. In the absence of a tone signal, cell discharges were always preceded by an EPSP. However, not every EPSP that depolarized the cell to its firing level was followed by spike initiation. For instance, during 15 sec of spontaneous activity, only 286 of the 350 EPSPs that depolarized the cell to its firing level were followed by a cell discharge. Low-intensity tone signals (up to 20 db SPL) did not alter the relationship between membrane potential and spike initiation, but changes appeared as the tone intensity was raised. At 90 db SPL, a level of depolarization sufficient to evoke discharges at approximately 100/sec during the best frequency signal (13 kHz) was ineffective in evoking even a single discharge with a tone only slightly removed in frequency (15 kHz). In addition, a small

spike (.5–1.5 mv in amplitude, < 1 msec in duration), distinct from the cell's larger spike (5 mv in amplitude, 1–2 msec in duration), appeared in the period after the tone termination. This smaller spike was never noted to have a preceding EPSP or to occur simultaneously with the large spike. When the cell was lost both spikes disappeared. Finally, the extent of the inhibitory surround, as defined by suppression of spontaneous discharges, involved a wider range of tonal frequencies than those eliciting IPSPs. The observations that with intense tonal signals spike firings might not be related to the level of evoked depolarization and that there could be suppression of spontaneous activity without observable IPSPs were also characteristic of the other 7 cells in which membrane potential and spike firings had a variable relation. However, the finding of additional

small spikes seemed to be a peculiarity of cell 20-6.

*Poststimulus time histograms and average membrane level*

In 21 units the pattern of discharges evoked by a tone stimulus (PSTH) was compared with the simultaneous changes in membrane level (averaged membrane level). The two measures were analyzed by visual inspection, taking into account the phase shift in the rise and decay portions of the averaged membrane levels introduced by the low-pass filter. The shapes of the PSTH and averaged membrane level seemed comparable whenever tones suppressed spontaneous activity and also evoked a sustained hyperpolarizing shift (Fig. 6). In contrast, firing pattern and membrane potentials had a variable relationship during excitatory tone bursts. In some cells the two measures had comparable shapes (left side Fig. 7, Fig. 8), whereas significant differences were apparent in responses obtained from other cells (right side Fig. 7). In the latter group for instance, the occurrence of cell discharges did not necessarily correlate with the level of membrane depolarization. Cell discharges might be initiated after a delay of 20–100 msec following tone onset and then increase gradually in frequency to a steady rate even though the membrane was depolarized at either the same level or in a gradually decreasing fashion throughout the tone burst. Features of the acoustic stimulus proved to be an important variable affecting the correspondence between firing pattern and averaged membrane level. In records from the same cell the two measures could be markedly different during one tonal frequency but well correlated if that same tone were reduced in frequency (cf. responses to 13 kHz in Fig. 5 to 60 db and 20 db), or if a different tonal frequency were presented (cf. 20-6, Figs. 5 and 6).

*Intracellular current stimulation*

Current stimulation was carried out on 16 cells recorded in a quasi-intracellular manner. Passage of current through the tip of the recording micropipette affected discharge rates in 12 of the cells tested: both spontaneous and tone-evoked activity

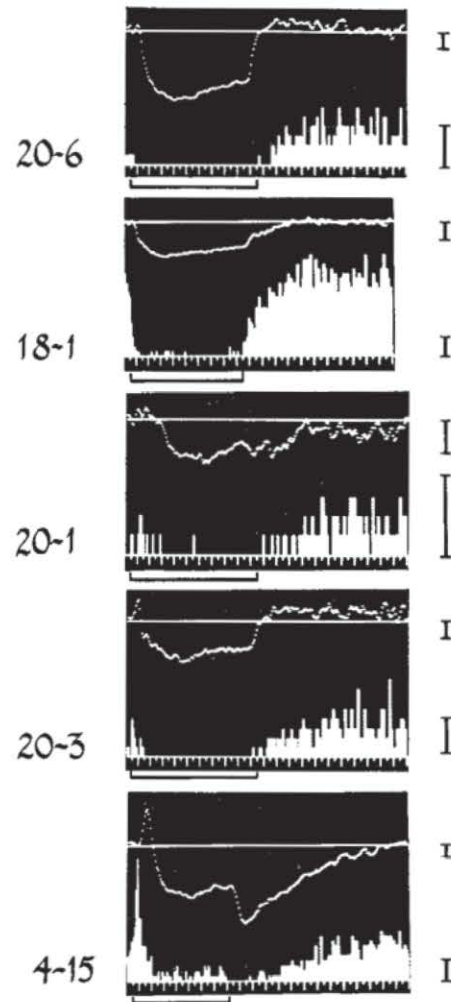


FIG. 6. Averaged membrane level (*upper portion* of each photograph) and poststimulus time histogram of spike discharges (*lower portion* of each photograph) for five cells during stimulation with tones that both suppressed spontaneous discharges and evoked a sustained hyperpolarizing shift. Forty tone bursts were used in the computation. Calibration for averaged membrane level is 1 mv and PSTH is four discharges. 20 msec separates the large marks on the abscissa of each photograph. Tone is indicated by the black line below each histogram.

increased with anodal currents and decreased with cathodal currents. Thresholds for affecting discharge rates ranged from 0.2 to 3 na and are comparable to current strengths used by other investigators for altering cell discharge rates in situations where the electrode is known to be in an intracellular position. In the present experiments cell discharges evoked by applied currents could be modified by the simultaneous presentation of tones. Frequencies that suppressed spontaneous activity also suppressed discharges evoked by the passage

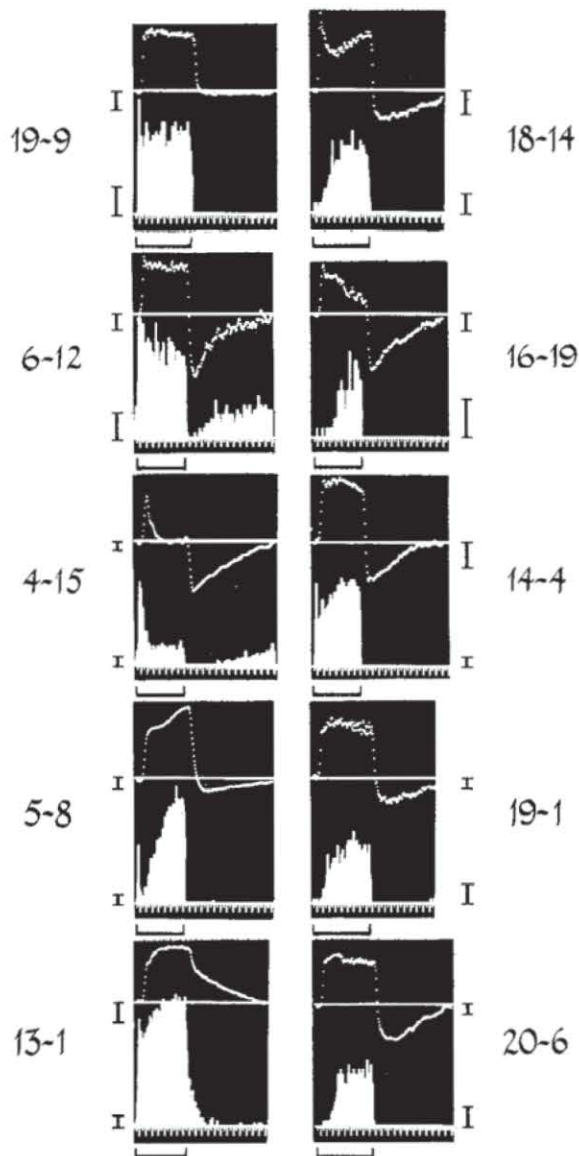


FIG. 7. Averaged membrane level (upper portion of each photograph) and poststimulus time histograms of spike discharges (lower portion of each photograph) for 10 different cells during stimulation with tones that both increased discharge rates and evoked a depolarization shift. Computations are based on 40 tone bursts except in cell 5-8, in which 200 trials were used. Calibrations as in Fig. 6, except the bar for average membrane level in cell 5-8 represents 2 mv.

of currents (Fig. 9). Furthermore, even in those cells without spontaneous activity, discharges evoked by anodal currents were also reduced in rate whenever tones adjacent to the excitatory frequencies were presented (Fig. 10). Thus, cellular excitability to injected currents is markedly reduced during the presentation of tones in the inhibitory surround. In cell 18-15, for instance, the threshold for firing the cell by

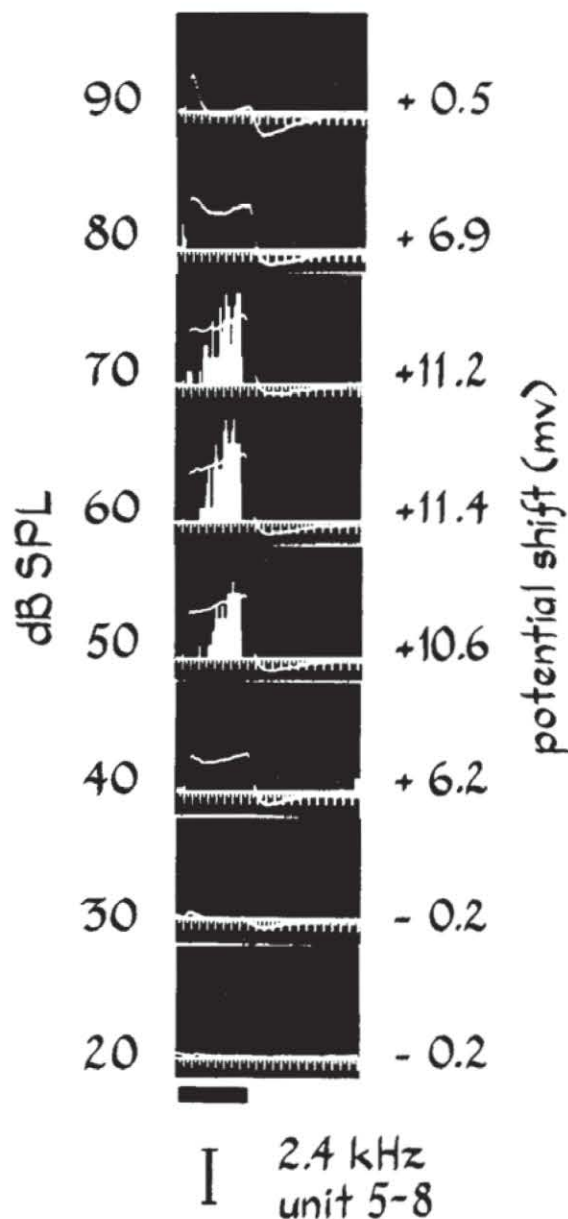


FIG. 8. Comparison of averaged membrane levels (continuous curves) and poststimulus time histograms (bars) of spike discharges for cell 5-8 measured at one tone frequency over a 70-db intensity range. Forty tone bursts were used in the computation of each intensity. Amplitude of the depolarizing shift measured at the end of the tone burst is indicated in the right column. Period of the tone is marked by the solid black bar at the bottom of figure. Time scale as in Fig. 6. Amplitude calibration, four spike discharges.

anodal currents increased from 1.2 na, in the absence of tonal stimulation, to 6 na when a tone in the inhibitory surround was concomitantly presented. Finally, the excitability of cells to anodal currents was also markedly depressed in the period immediately following tone offset when both



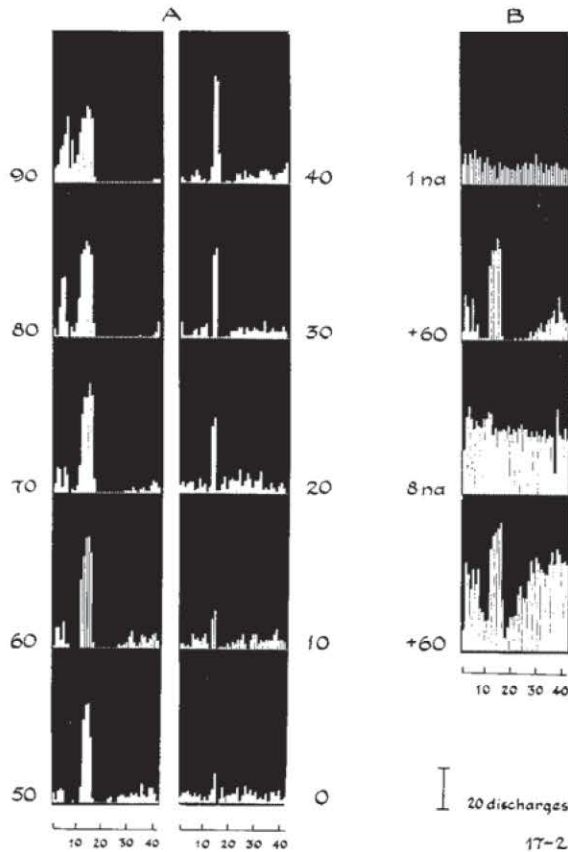


FIG. 9. Response-area histograms for cell 17-2 over a 90-db sound intensity range (*A*). Numbers along the abscissa represent 42 discrete tonal signals covering the frequency range between 1.5 and 22.0 kHz, and numbers to the side of the response-area histogram indicate the tone intensity in db SPL. Each bar in the histogram represents the total number of spikes evoked by one tone frequency. In *B*, discharge rates during two different strengths of depolarizing currents are shown in the photographs labeled 1 na, 8 na and are to be compared with discharge rates when these same currents were applied during the tonal signals at 60 db (+60). Note the decreased rate of current-evoked discharges when tones in the inhibitory surround (*f* 8-11, 5-6.5 kHz and *f* 17-26, 9.5-14 kHz) were presented.

spontaneous activity was suppressed and the membrane hyperpolarized.

#### DISCUSSION

The present experiments show excitatory and inhibitory synaptic events in cells in the cochlear nucleus during acoustic stimulation. Depolarizing shifts accompanied tones that increased firing rate, and hyperpolarizing shifts accompanied tones that suppressed activity. The classification of the hyperpolarizing response as an inhibitory postsynaptic potential is suggested by the marked increase in intracellular depo-

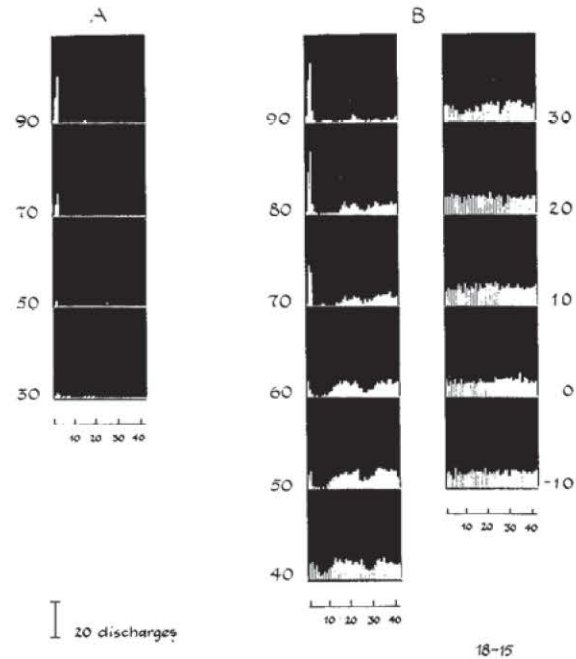


FIG. 10. Effect of steady depolarizing current (*B*) on response-area histogram (*A*) in a cell with very low rates of spontaneous activity ( $< 1/\text{sec}$ ). Three na of depolarizing current increased discharge rate to approximately 50/sec. Two distinct bands of tonal frequencies can be seen to decrease the rate of current-evoked discharges. The first extending from *f* 4-9, 3-5.5 kHz encompassing frequencies adjacent to the unit's excitatory response area; the second, extending from *f* 26-32, 14-17 kHz seen best with tone intensities between 20 and 60 db SPL.

larizing currents required to fire the cell during the hyperpolarizing shift compared to the current levels needed to fire the cell in the resting state (7). These results complement Gerstein, Butler, and Erulkar's (6) recent intracellular study in cochlear nucleus of cat in which IPSPs were not seen though the inhibitory effects of tones were apparent from discharge patterns and spike-count data. Our results attest to the difficulties of recording IPSPs in cochlear nucleus. Of the 29 cells in which discharges were suppressed by tones, IPSPs could be detected in only 16. Furthermore, even in these 16 cells, IPSPs were not seen with every tone that suppressed spontaneous activity though the inhibitory postsynaptic effects of these tones were apparent from the marked depression in responsiveness to intracellular depolarizing currents that accompanied their presentation (7).

The failure to detect IPSPs suggests that the recording electrodes were remote from

the zone of spike generation and thereby were unable to accurately sample all of the synaptic events governing spike initiation. This interpretation could also account for the frequent inconsistencies in firing levels noted for these cells. An electrode remote from the zone of spike generation can provide information about the distribution of excitatory and inhibitory synapses on the cell surface. The finding that depolarizing shifts almost invariably accompanied tones that increased firing rates (43 of 49 cells) suggests that excitatory synapses are widely distributed over the cell surface and can be detected by an electrode almost anywhere in the cell. Inhibitory synapses, on the other hand, appear to be restricted to regions close to the spike generator, as suppression of discharges was frequently noted in the absence of a recordable hyperpolarizing shift. Furthermore, depolarizing shifts were not always associated with spike discharges, whereas hyperpolarizing responses were, without exception, accompanied by a suppression of cell firing. A differential distribution of excitatory and inhibitory input has been defined for the anterior horn cell of the cat with inhibitory synapses located close to the cell soma but excitatory synapses distributed over both the soma and dendrites (19). In the cochlear nucleus the occurrence of inhibitory synapses close to the spike-generating region would be an efficient means for restricting the cell's acoustic response characteristics. Moreover, some of the patterning of spike discharges demonstrated for cells in this nucleus (15) could be accounted for by temporal characteristics of the inhibitory input. For instance, in the present experiment units that had a burst of discharges at tone onset followed by a brief period of silence and then sustained firings showed depolarizing shifts to tones that were interrupted shortly after onset by an IPSP of short duration.

The present experiments show that inhibition in the cochlear nucleus is clearly postsynaptic but provide no explanation as to the origin of the inhibitory input. It is apparent that inhibition has a well-defined relationship to the cell's acoustic response area. This specificity could derive from inhibitory interneurons within the nucleus

that are responsive to a limited range of acoustic stimuli, or from VIIIth nerve fibers themselves that are inhibitory in action.

#### *Aftereffects of sound*

Cells in the cochlear nucleus have a marked suppression of spontaneous activity following sound stimulation that can persist for many seconds (21) or even minutes (20). It is not known whether the change in spontaneous activity is due to alternations in afferent input since VIIIth nerve fibers also show a suppression of activity following tone stimulation (10), or whether the cochlear nucleus changes are mediated centrally. The finding in the present experiments of hyperpolarization in cochlear nucleus cells at tone offset when spontaneous activity was suppressed is evidence in support of central neuronal mechanism for this aftereffect. Furthermore, the observation that discharges evoked by intracellular depolarizing currents were also suppressed at tone offset indicates that the excitability change occurs at the level of the cochlear nucleus cells themselves. The hyperpolarizing shift at tone offset could represent a change in permeability to  $\text{Cl}^-$  or  $\text{K}^+$  ions as occurs typically in IPSPs (3) or to  $\text{Na}^+$  ions as occurs following activation of the electrogenic sodium pump (1). The latter mechanism has been implicated for hyperpolarization and inhibition following a period of intense stimulation that occurs in invertebrate receptors (13), invertebrate central nervous system neurons (14, 16), and mammalian C fibers (17). The observations, in the present experiments, that hyperpolarization at tone offset increased in amplitude as tone intensity was raised and occurred in cells in which IPSPs to inhibitory tone bursts could not be detected raise the possibility that an electrogenic sodium pump might participate in its generation. Our data cannot distinguish between the alternative ionic mechanisms for hyperpolarization at tone offset as no attempt was made to measure the equilibrium potential or to determine the effects of intracellular injections of appropriate ions. In either case, hyperpolarization at tone offset is a powerful means of regulating spontaneous activity and may be the neural

equivalent of some of the perceptual after-effects of sound stimulation.

#### SUMMARY

Intracellular and quasi-intracellular recordings were made from 58 cells in the cochlear nucleus of pentobarbital-anesthetized cats during the presentation of tone bursts covering the frequencies from 0.5 to 22.0 kHz and intensities up to 90 db SPL.

In some cells there was a good correspondence between membrane potential changes and spike firings. Depolarizing shifts accompanied tones that increased discharge rates, and hyperpolarizing shifts accompanied tones that suppressed activity. The configuration of the membrane potential changes closely resembled the temporal patterning of spike discharges. In other cells membrane potential changes often failed to correspond to cell discharges. Suppression of activity could occur without a detectable hyperpolarizing shift, and the shape of the depolarizing shifts was often at variance with the pattern of cell activity. It is assumed that the electrode, in the latter group of cells, was in a site remote from the zone of spike generation.

Passage of intracellular currents defined that tones in the inhibitory surround always effected a marked depression in cel-

lular excitability even in the absence of a hyperpolarizing response.

These results suggest that excitatory and inhibitory synaptic inputs are distributed differently in cochlear nucleus cells: inhibitory inputs occurred principally near the zone of spike generation, so their effects may not be detected by an electrode in a remote portion of the cell, whereas excitatory synapses are widely distributed over the cell surface.

Three types of membrane potential changes were noted at tone offset: 1) hyperpolarization, 2) hyperpolarization followed by a rebound depolarization, and 3) a sustained depolarization. Hyperpolarization at tone offset occurred most frequently and was accompanied by a suppression of spontaneous activity and a decrease in responsiveness to stimulation with intracellular depolarizing currents.

#### ACKNOWLEDGMENTS

We express our appreciation to Birthe Nyholm for her illustrations and Pamela Barrett for secretarial assistance.

This work was supported by Grant NB-05700 from the National Institutes of Health. Presented in brief at the April, 1969, meeting of American Neurological Academy, Washington, D.C.

A. Starr was recipient of Career Development Award NB-31242 from National Institute of Neurological Diseases and Stroke.

R. Britt was recipient of Life Insurance Medical Research Fund Fellowship MS-69-2.

#### REFERENCES

- ADRIAN, R. H. AND SLAYMAN, C. L. Pumped movements of K and Rb in frog muscle. *J. Physiol., London* 175: 49P-50P, 1964.
- ARAKI, T. AND OTANI, T. Response of single motoneurons to direct stimulation in toad's spinal cord. *J. Neurophysiol.* 18: 472-485, 1955.
- ECCLES, J. C. *The Physiology of Synapses*. New York: Academic, 1964.
- ERULKAR, S. D., BUTLER, R. A., AND GERSTEIN, G. L. Excitation and inhibition in cochlear nucleus. II. Frequency-modulated tones. *J. Neurophysiol.* 31: 537-548, 1968.
- GALAMBOS, R. AND DAVIS, H. Action potentials from single auditory-nerve fibers. *Science* 108: 513, 1948.
- GERSTEIN, G. L., BUTLER, R. A., AND ERULKAR, S. D. Excitation and inhibition in cochlear nucleus. I. Tone-burst stimulation. *J. Neurophysiol.* 31: 526-536, 1968.
- GRANIT, R., KELLERTH, J.-O., AND WILLIAMS, T. J. 'Adjacent' and 'remote' post-synaptic inhibition in motoneurons stimulated by muscle stretch. *J. Physiol., London* 174: 453-472, 1964.
- GREENWOOD, D. D. AND MARUYAMA, N. S. Excitatory and inhibitory response areas of auditory neurons in the cochlear nucleus. *J. Neurophysiol.* 28: 863-892, 1965.
- KATSUKI, T., WATANABE, T., AND SUGA, N. Interaction of auditory neurons in response to two sound stimuli in cat. *J. Neurophysiol.* 22: 603-623, 1959.
- KIANG, N., WATANABE, T., THOMAS, E. C., AND CLARK, L. F. *Discharge Patterns of Single Fibers in Cat's Auditory Nerve*. Cambridge, Mass.: MIT Press, 1965.
- McILWAIN, J. T. AND CREUTZFELDT, O. D. Microelectrode study of synaptic excitation and inhibition in the lateral geniculate nucleus of the cat. *J. Neurophysiol.* 30: 1-21, 1967.
- MOUSHEGIAN, G., RUPERT, A., AND GALAMBOS, R. Microelectrode study of ventral cochlear nucleus of the cat. *J. Neurophysiol.* 25: 515-529, 1962.
- NAKAJIMA, S. AND TAKAHASHI, K. Post-tetanic hyperpolarization and electrogenic Na pump in stretch receptor neurone of crayfish. *J. Physiol., London* 187: 105-127, 1966.
- NICHOLLS, J. G. AND BAYLOR, D. A. Long-

- lasting hyperpolarization after activity of neurons in leech central nervous system. *Science* 162: 279-281, 1968.
15. PFEIFFER, R. Classification of response patterns of spike discharges for units in the cochlear nucleus: tone burst stimulation. *Exptl. Brain Res.* 1: 220-235, 1966.
  16. PINSKER, H. AND KANDEL, E. R. Synaptic activation of an electrogenic sodium pump. *Science* 163: 931-935, 1969.
  17. RANG, H. P. AND RITCHIE, J. M. On the electrogenic pump in mammalian non-myelinated nerve fibres and its activation by various external cations. *J. Physiol., London* 196: 183-221, 1968.
  18. RUPERT, A., MOUSHEGIAN, G., AND GALAMBOS, R. Unit responses to sound from auditory nerve of cat. *J. Neurophysiol.* 26: 449-465, 1963.
  19. SMITH, T. G., WUERKER, R. B., AND FRANK, K. Membrane impedance changes during synaptic transmission in cat spinal motoneurons. *J. Neurophysiol.* 30: 1072-1096, 1967.
  20. STARR, A. AND LIVINGSTON, R. B. Long-lasting nervous system responses to prolonged sound stimulation in waking cats. *J. Neurophysiol.* 26: 416-431, 1963.
  21. STARR, A. Suppression of single unit activity in cochlear nucleus of the cat following sound stimulation. *J. Neurophysiol.* 28: 850-862, 1965.
  22. TASAKI, I. Nerve impulses in individual auditory nerve fibers of guinea pig. *J. Neurophysiol.* 17: 97-122, 1954.