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Synaptic Events and Discharge Patterns of Cochlear Nucleus Cells. I. Steady-Frequency Tone Bursts

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THE COCHLEAR NUCLEUS consists of several subdivisions with different neuronal morphology and synaptic relations to incoming eighth nerve fibers (1, 10, 11, 16, 19–21). There is a corresponding uniqueness of unit activity to tonal signals in these different regions (6, 12, 13, 22). Pfeiffer (22) characterized the differences in terms of both spontaneous and evoked activity to short-duration tones (25 ms) presented close to threshold intensity. He noted four classes of cells in cochlear nucleus and provided names that were descriptive of the peristimulus time histogram or PSTH pattern: 1) primarylike, resembling eighth nerve fibers, found in both posterior ventral and anterior ventral cochlear nucleus; 2) chopper, found in ventral and dorsal cochlear nucleus; 3) pause, found only in dorsal cochlear nucleus; and 4) on, found in ventral and dorsal cochlear nucleus. Primarylike units have a high level of activity. Chopper units show regular-spaced peaks of activity through the tone burst. Pause units show an onset burst, then a pronounced dip in firing, and a gradual resumption of sustained activity. And finally, on-units that only discharge at tone onset. Pfeiffer's classification is affected by signal characteristics. For instance, the chopper category cannot be distinguished if tone duration is lengthened to several hundred milliseconds. Moreover, if signal intensity is raised, a new type of response pattern, characterized by long latency (> 10 ms) from tone onset to the appearance of unit activity, is apparent (6, 9, 28). Inclusion of other features, such as suppression of activity after tone offset and both the presence and extent of tone-evoked suppression of spontaneous activity, termed inhibitory surround, extends the classification still further (6).

The synaptic bases for the different response patterns is not known. Intracellular measures in cochlear nucleus are few (8, 29, 30) and have

thus far revealed depolarizing shifts during tones that increase unit activity and hyperpolarizing shifts during tones that suppress activity. In this paper we will examine the synaptic events underlying the different discharge patterns of cochlear nucleus cells to steady-frequency tone bursts, 250 ms in duration, at several different frequencies and intensities within the response area. The results are pertinent for an accompanying paper (3) in which intracellular and unit activity to frequency-modulated acoustic signals are studied and correlated with the different response patterns to steady-frequency tones.

METHODS AND PROCEDURES

Surgical preparation

Forty-five adult cats weighing between 1.8 and 3 kg were anesthetized with sodium pentobarbital (Nembutal) (40 mg/kg). Supplemental injections were administered as needed. Body temperature was monitored and maintained at approximately 37°C by a heating jacket. The preparation of the animal included a tracheotomy and a cutdown of the left saphenous vein. The animal's head was placed in a face clamp attached to a stereotaxic frame and the body supported in a hammock.

An incision was made inferior to the right pinna and the bulla exposed. A hole was made in the bulla and an insulated stainless steel wire with an exposed ball tip was placed on the round window for monitoring cochlear microphonic and attached to the side of the bulla with acrylic cement. A right posterior fossa craniectomy was performed and a portion of the right cerebellar hemisphere aspirated to expose the right cochlear nucleus and eighth cranial nerve. The right pinna was removed and a short hollow tube (0.65 cm wide, 2.5 cm long) inserted into the auditory canal so that its end was within a few millimeters of the tympanic membrane. The opposite end of the tube was

flared to accept the acoustic transducer, a $\frac{1}{2}$ inch Brüel & Kjaer microphone.

The animal was artificially respired (stroke volume of 50 ml and a rate of 24 cycles/min) and paralyzed with gallamine triethiodide (Flaxedil) delivered intravenously at a rate of 1 ml/h using a Harvard infusion pump. No measures of blood pressure or expired PCO_2 were made. Bilateral pneumothoraxes were performed. These were necessary to reduce pulsation from venous pressure fluctuations associated with the respiratory cycle. It was usually necessary to elevate the head of the animal to reduce pulsations. In at least one-fourth of the animals sufficient pulsations still occurred and interfered with data collection. After surgery the animal was moved into the sound-attenuating room where a determination of the sound intensity to evoke 100 μV of cochlear microphonic was made for tonal signals from 100 Hz to 40 kHz. Cochlear microphonic amplitudes were measured periodically through the experiment to check on the constancy of the acoustic input.

Sound signals

The signals presented were clicks and tone bursts. Clicks were produced by applying a 150-V, 0.3-ms square wave to the transducer. The transducer was polarized with a DC voltage of 200 V for tone stimulation. Sinusoidal voltages (40 V peak to peak) for the tone bursts were generated by a voltage-controlled oscillator (Hewlett-Packard 3300A function generator with a 3,350 A sweep module) passed through an attenuator for regulation of intensity and then through an electronic switch for regulation of rise and decay times (5 ms). The tone-burst duration was 250 ms with 250 ms of silence before the next tone burst. The tone bursts could be presented at a single pre-selected frequency or as a program of 40 different frequencies that ascended in a stepwise manner. The frequency of the initial tone burst was specified, and subsequent tone bursts ascended in frequency as integral multiples of the initial tone frequency. The most common ranges covered were 0.1–4 kHz (100-Hz intervals) and 0.5–20 kHz (500-Hz intervals). For details of the programming see Britt's thesis (2).

Intensity of all acoustic signals was adjusted in 10-dB steps below the maximum level. The sound-pressure level generated by this system was determined at the beginning of the experiments by fitting a $\frac{1}{4}$ inch Brüel & Kjaer microphone at the end of the hollow tube which would be close to the tympanic membrane and measuring the signal levels with a B and K

sound-level meter. At maximum input (-0 dB *re* transducer input), the sound output was 100 ± 10 dB from 0.3 to 40 kHz. (See Fig. 1.)

Data acquisition

Glass micropipettes filled with 2 M potassium citrate with impedances of 20–25 $M\Omega$ (tested at 60 Hz with a Winston electrode impedance meter) were found to be most satisfactory. A WPI pre-precision electrometer was used as the negative-capacitance unity gain amplifier and to pass current through the tip of the micropipette. The output of the electrometer was amplified 50- or 100-fold using a noninverting operational amplifier and displayed on an oscilloscope and recorded on magnetic tape at 30 inches/s. The frequency response of the recording system was linear between DC and 10 kHz.

Experimental procedure

The glass micropipette was placed in an electrode drive (Trent Wells) and positioned just over the cochlear nucleus with the aid of an operating microscope. The drive was subsequently advanced remotely from outside the sound-attenuating room. In five animals the electrode was positioned over the eighth nerve. Once the electrode was lowered to the surface, liquid isoosmotic agar-agar close to 37°C temperature was poured into the cavity surrounding the electrode and cochlear nucleus to dampen cardiorespiratory pulsations.

Clicks (25/s) were presented as the electrode was lowered through the cochlear nucleus. When a unit was encountered that was either spontaneously active or responsive to the click, its depth along the pass was noted and a tuning curve defined by presenting a series of 40 tone bursts in ascending frequency at several different intensities. FM signals were then presented (see ref 3 for details and results). Next, 40 trials of various single tone frequencies (usually the

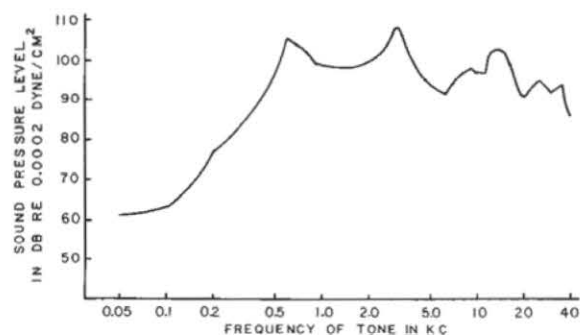


FIG. 1. Sound-pressure level (SPL) output of the acoustic system as a function of signal frequency (0.05–40 kHz). 0 dB reference level was a 40 V p-p sinusoidal voltage applied to the transducer, which was polarized with 200 V.

best frequency and those in the inhibitory surround) were presented for computer-generated peristimulus timed histograms (PSTH). Finally, we attempted to pass current through the microelectrode during tone presentation to establish equilibrium potentials for various synaptic events. Most units were lost before the entire protocol was completed.

Extracellular recordings of single units were characterized by biphasic spikes and little or no change in the DC level. We did not note whether there was a notch on the ascending limb of the spike which is supposedly characteristic of cells in anterior ventral cochlear nucleus (23). The electrode was considered intracellular or quasi-intracellular (17) if there was a drop in the DC level and cell discharges were of a positive polarity. The spikes of nerve fibers were distinguished by their sharp rise time, positive polarity without membrane-potential shifts. We attempted to define the tuning curve and FM responses when the electrode was extracellular or quasi-intracellular before advancing the electrode to the intracellular position. Stable intracellular recordings could only be maintained for a few minutes.

Data analysis

The tape-recorded data was photographed using a Grass (C4) camera to produce film strips for visual examination. Where appropriate the following computer analyses were made using a LINC computer: 1) Response area histogram: number of discharges both during each 250-ms tone burst of the tuning curve (excitatory response-area histogram) and during the subsequent 250 ms after the tone burst (off-count histogram) were computed. 2) Peristimulus time histogram (PSTH): the number of discharges occurring during sequential 2-ms epochs of a 250-ms tone burst were determined during 40 trials of one tonal frequency. 3) Average membrane potentials: an analysis of membrane slow potentials was obtained by attenuating the spikes using a low-pass filter (Allison filter) with its 3-dB down point set at 75 Hz and averaging the filtered signal. For tone bursts 40 trials were used. With this program random membrane fluctuations cancel out.

The LINC computer was programmed to compute the PSTH and the averaged membrane-potential determination simultaneously. Polaroid pictures were taken of each PSTH with its corresponding averaged membrane potential.

Localization of units

Precise localization of units was not possible in this study. The visualization of the electrode as it entered the nucleus allowed a gross esti-

mate as to whether we were anterior or posterior in the structure. The tip of the glass electrode was broken off in three of the animals and histological sections were made after fixation to see the subdivisions of the cochlear nucleus the electrode tract had traversed.

RESULTS

A total of 164 cochlear nucleus cells and 24 eighth nerve fibers were studied. Significant membrane changes were obtained in 55 of the 164 cochlear nucleus cells. Four examples of each of the various response categories are shown in Fig. 2.

Eighth nerve fibers have a single response pattern to tone bursts at their characteristic frequency: there is an onset burst of spikes followed by a smooth decrease to a relatively stable rate of discharge (first column, Fig. 2). In contrast, we could distinguish four major response patterns to tone bursts at the characteristic frequency in cells of cochlear nucleus. The terms employed are descriptive of the PSTH patterns and differ in several aspects from Pfeiffer's (22) classification because of differences in tone duration (25 ms for Pfeiffer versus 250 ms in this study) and in tone intensity (within 20 dB of threshold for Pfeiffer versus suprathreshold in this study).

1) Primarylike units responded in a manner identical to eighth nerve fibers (Fig. 2, second column). Discharges were maximal at tone onset and then decreased in a smooth manner till a steady discharge rate was assumed; 33 primary units were studied with characteristic frequencies ranging from 0.1 to 22 kHz. They were detected in rostral penetrations of the nucleus.

2) Buildup units were characterized by the gradual increase of activity during the tone burst. Most showed an onset burst, followed by a 10- to 30-ms period of suppressed firing, and then a gradual buildup of activity for the remainder of the tone burst. The onset response could be small (unit 24-2, Fig. 2) or even undetectable at certain signal intensities (unit 25-5, Fig. 2). Pfeiffer, in his study with short tone bursts, classified these units as "pause" types because the period of suppressed firing was the prominent feature rather than the subsequent gradual buildup of firing seen with long duration signals. Twenty-five buildup units were studied with characteristic frequencies ranging from 0.8 to 12.5 kHz.

3) Onset units discharge primarily at tone onset with little or no activity for the remainder of the tone burst (Fig. 2, fourth column); 13 onset units were studied with characteristic frequencies ranging from 0.1 to 12.0 kHz.

4) Pause units have a long latency of 10-60

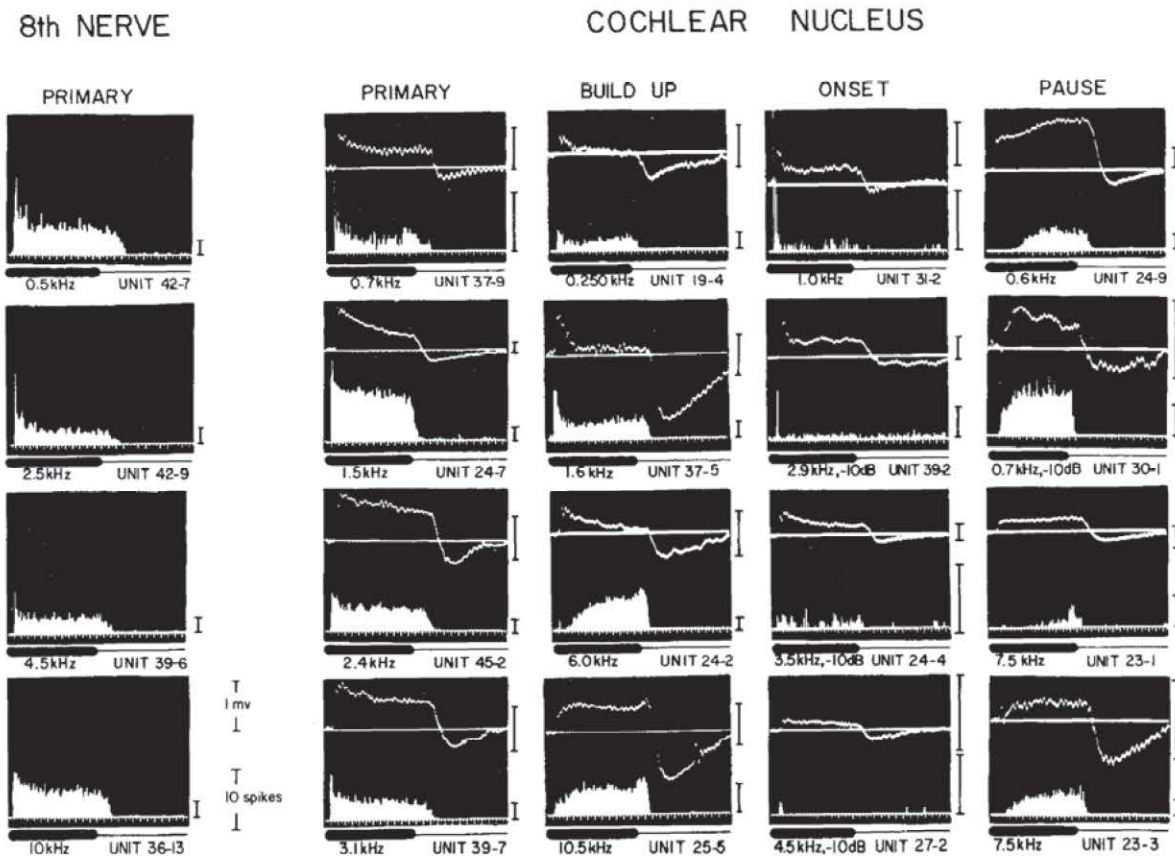


FIG. 2. Classification of eighth nerve fibers and cochlear nucleus cells on the basis of peristimulus time histograms (PSTHs) at the characteristic frequency. The eighth nerve response pattern to tone bursts is seen on the left. Cochlear nucleus units can be divided into four main types (primary or primarylike, buildup, onset, and pause). Upper portion of each photograph is the averaged membrane potential and the lower portion is the PSTH of the spike discharges. Forty tone bursts were used in each computation. Calibration for average membrane level is 1 mV and PSTH of spike discharges is 10 spikes; 20 ms separate the large marks on the abscissa of each photograph. In this and all subsequent figures, the black bar under each photograph represents the approximate timing for the tone burst. The onset of the tone burst began 15 ms and terminated 265 ms from the origin. The characteristic frequency, unit number, and intensity (if other than -0 dB) are listed under each photograph.

ms from tone onset to the appearance of spike discharges, compared to the usual 3–5 ms latency observed in the other response categories. Once activity was initiated, there was a gradual increase in firing till a maximal rate was achieved. The major distinguishing feature between buildup and pause units was the long latency of the latter group, 13 pause units were studied with characteristic frequencies ranging from 0.6 to 15.5 kHz. These units were recorded only in caudal penetrations of the nucleus.

Effects of tonal frequency and intensity on discharge patterns and membrane potentials

PRIMARYLIKE CELLS. The discharge patterns of primarylike units were similar to all tonal frequencies and intensities within the unit's response area except that activity was maximal to

tones near the characteristic frequency (Fig. 3). As signal intensity was raised above threshold, there was a corresponding broadening of the tuning curve and an increase in the number of discharges evoked by tones at the characteristic frequency without any change in the PSTH shape (Fig. 4).

Membrane potentials corresponded both in shape and amplitude with the configuration of the discharge pattern. Depolarization was maximal at tone onset, and then decreased in a smooth fashion to a steady level for the duration of the tone. In no instance was tone-evoked suppression of spontaneous activity observed nor was there membrane hyperpolarization to tonal frequencies adjacent to the characteristic frequency (Fig. 3).

At tone offset, in approximately one-half of the units (15 of 35), there was a suppression of unit activity below spontaneous rates and a cor-

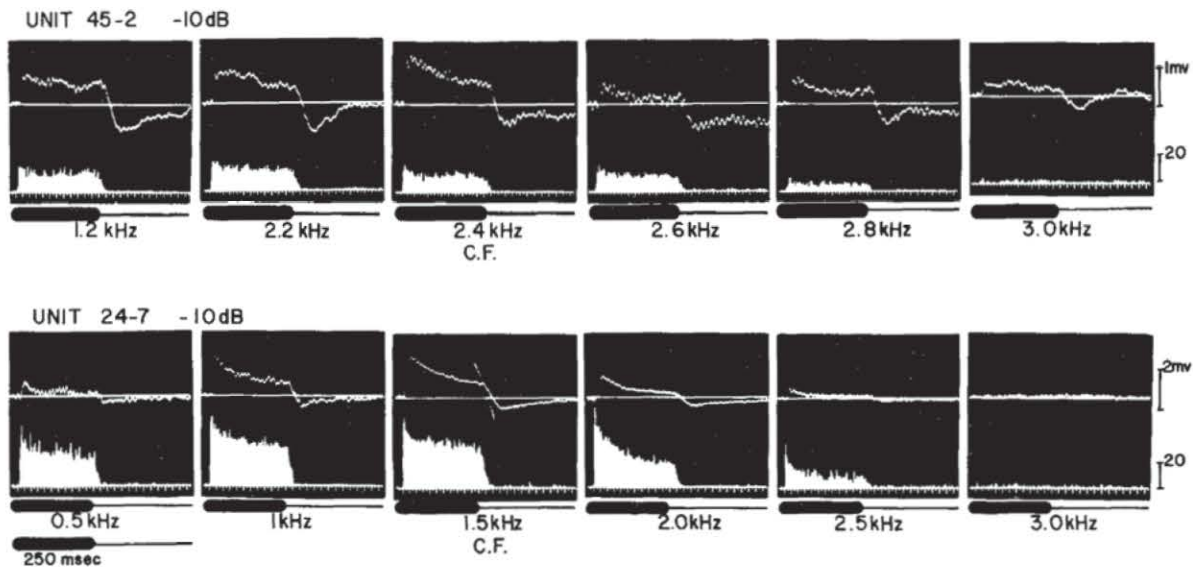


FIG. 3. Peristimulus time histogram (PSTH) at -10 dB as a function of signal frequency for two primary cells. The characteristic frequency (CF) is noted under the appropriate PSTH. The averaged membrane potential is the upper portion of each photograph and the PSTH of spike discharges is below. Forty tone bursts were used in each computation. Calibration for averaged membrane level is 1 mV for unit 45-2 and 2 mV for unit 24-7 and calibration for the PSTH is 20 spikes for both units. Note that primarylike configuration remains constant across the frequency response area except for increased number of unit discharges near the characteristic frequency.

responding hyperpolarization of the membrane, called off-hyperpolarization (Fig. 4). Both the duration and amplitude of these changes increased as signal intensity was raised. To intense signals (-0 dB) both the membrane depolarization and unit activity evoked by the tone burst would persist for 10 – 30 ms beyond tone offset, thereby delaying the appearance of off-hyperpolarization. Furthermore, in three units the off-hyperpolarization and suppression of unit activity was only transient and was followed by a resumption of high rates of activity and membrane depolarization, which we call off-excitation (Fig. 4, unit 42-4, -0 dB and Fig. 10, unit 24-1 containing only the PSTH data). It should be stressed that off-excitation was only observed in tones at the characteristic frequency and at the most intense levels (-0 dB *re* transducer input or 100 dB SPL).

BUILDUP CELLS. There was considerable variation in the configuration of the discharge patterns of buildup cells to tones at the characteristic frequency (see Fig. 2). Their common feature was the gradual increase in rate of firing from a point 10 – 30 ms after tone onset when activity was minimal, till tone offset when the rate could be as much as 4 times more rapid. In the same unit several different discharge patterns could be defined. For example, unit 19-7 in Fig. 5 had a sustained response to tones at 100 Hz, a buildup pattern to tones of 200 and 300 Hz, and an onset pattern to tones of still

higher frequency. This cell had the most diversified number of response patterns among the population studied. In other buildup units the extent of the onset burst of activity varied with changes in signal frequency (unit 25-6, 37-4, Fig. 5). Changes in tone intensity at the characteristic frequency revealed the buildup pattern most clearly when signals were from 10 – 40 dB above threshold. Unit 29-1 in Fig. 6 had a sustained response at threshold (-60 dB) and a buildup pattern only when signals were 40 dB more intense (-20 dB). A similar effect of intensity on the buildup pattern can be seen in the other two units in Fig. 6.

Examination of membrane events during tonal stimulation revealed the presence of several inhibitory events in buildup units. First, tonal frequencies adjacent to the response area produced an inhibitory surround: there was a suppression of discharge and an accompanying hyperpolarization of the membrane (unit 25-5, 25-6, 37-4, Fig. 5). Furthermore, during the presentation of tones within the excitatory response area that evoked the buildup pattern there could be a brief unsustained period of membrane hyperpolarization shortly after tone onset associated with a suppression of firing (see unit 19-7 to 0.2 – 0.9 kHz and unit 25-6 to 1.1 kHz, Fig. 5). There were other buildup units in which the correlation between membrane events and discharge patterns was not as close (see Fig. 2, unit 24-2), suggesting that the electrode was at a site remote from the spike-

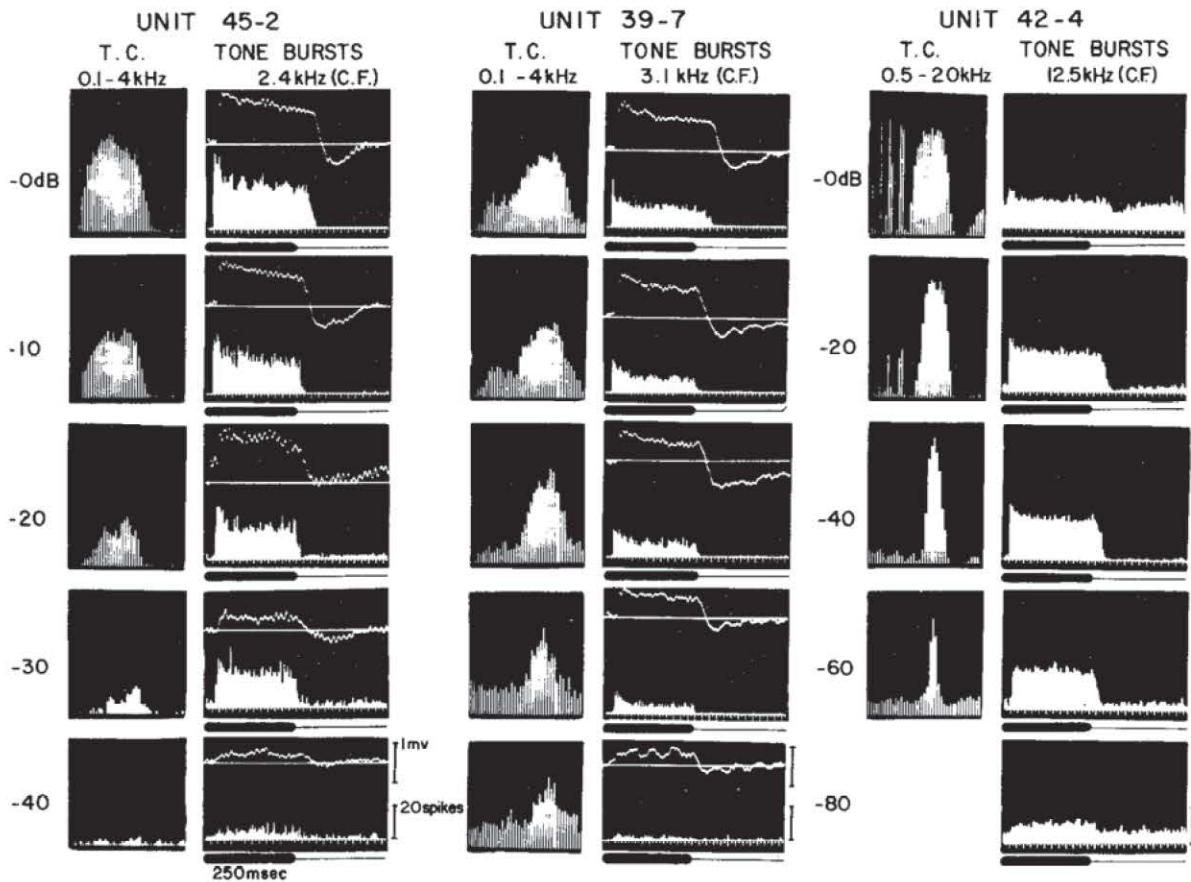


FIG. 4. Effects of intensity on the frequency response area or tuning curve (TC) and peristimulus time histogram (PSTH) for three primarylike cochlear nucleus cells. Averaged membrane potential (upper portion of each PSTH photograph) is shown for units 45-2 and 39-7. Calibrations are 1 mV for the averaged membrane potential and 20 spikes for TC and PSTH. Note the maintenance of the primarylike pattern across signal intensity. There is a persisting discharge after tone offset as signal intensity is raised and, in addition, in unit 42-4 there develops an off-excitation at -0 dB.

generating region (29). In summary, there is evidence in this group of cells that active inhibition, manifested by membrane hyperpolarization and suppression of discharges, plays a prominent role in both the genesis of the build-up response to a steady tone burst and the occurrence of the inhibitory surround.

ONSET CELLS. The distinguishing characteristic of onset cells is the restriction of discharges principally to the period immediately following the tone's commencement. In the primarylike cells, responses are also maximal at tone onset, but then decrease in a smooth manner to some lower level of activity. In contrast, onset cells have an abrupt decrement in firing within 20 ms after tone onset to a very low level of activity. The onset pattern was observed to all signals within the unit's response area (Fig. 7). Furthermore, changes in signal intensity over a 40-dB intensity range did not alter the onset characteristic (Fig. 8).

Examination of membrane changes in onset cells revealed that depolarization was maximal

at tone onset and then decreased either in a gradual manner (unit 24-4, Fig. 7, unit 27-2, Fig. 2) or more precipitously (unit 31-2, Fig. 7, unit 39-2, Fig. 2) to a lower but sustained level of depolarization. Thus, during the tone burst, onset units are obviously receiving synaptic drive sufficient to depolarize the membrane but insufficient to evoke frequent cell discharges. Some of these cells also provide evidence of the presence of active inhibition by membrane hyperpolarization to tones in the inhibitory surround. Moreover, in unit 39-2, Fig. 7, the first membrane event to tones from 0.8 to 2.8 kHz is a brief depolarization, followed either by a marked hyperpolarization (0.8, 0.9, and 2.6 kHz) or merely a return to the resting level (2.7 and 2.8 kHz). It may be that the development of an onset pattern depends on the temporal sequence of excitation followed by more powerful and sustained inhibition.

PAUSE CELLS. The discharge pattern was characterized by a long latency (> 10 ms) between tone onset and unit responses. In these

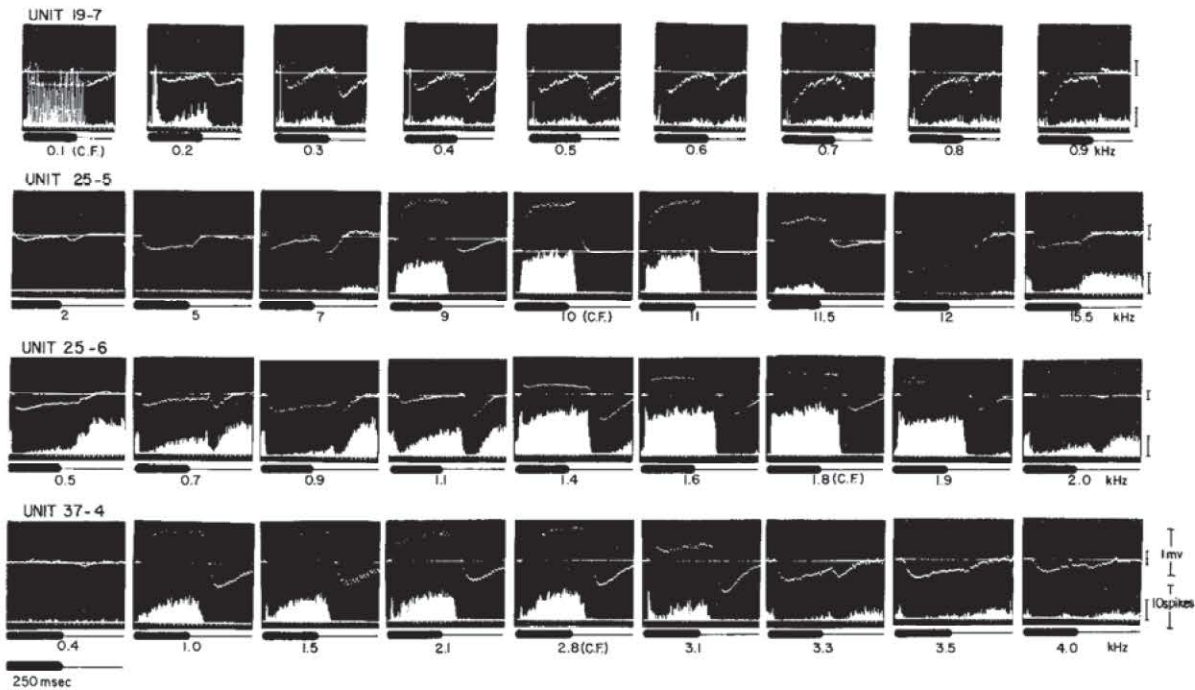


FIG. 5. Peristimulus time histogram (PSTH) at -10 dB as a function of signal frequency in buildup cells. Note that the PSTH configuration of unit 19-7 gradually changes from buildup configuration to an onset response as frequency increases. Unit 25-5 shows tone-evoked membrane hyperpolarization (inhibitory surround) on both the low- and high-frequency ends of the frequency response area. Unit 25-6 shows inhibition at the low-frequency end of the unit's response area. Note the change from buildup response at the characteristic frequency to a sustained level of firing at the characteristic frequency. Unit 37-4 has a high-frequency inhibitory surround with membrane hyperpolarization.

units, changes in the tone's frequency within the response area had a significant effect on the configuration of the PSTH (Fig. 9, unit 24-1). At the fringes of the response area (5.5, 10.5, 11.0 kHz, Fig. 9), the PSTH configuration is primary in nature. As the frequency moves closer to the characteristic frequency, the latency or pause pattern develops. Signal intensity also had a dramatic effect on the response pattern (Fig. 10). At threshold the unit responded at short latency with a sustained response (-80 dB, Fig. 10, right column). As intensity was raised, a delay or pause in unit discharge appeared which became maximal 60 dB above threshold (-20 dB, Fig. 10, right column). The mechanisms generating the pause were even capable of suppressing the off-excitation discharges to tones of high intensity. (Note the depressed firing between the first and second vertical time-base marks in the 0-dB histogram, Fig. 10, right column.)

The membrane events accompanying the pause pattern either showed, 1) a slightly smaller depolarization during the pause than later in the tone burst when the unit discharged vigorously (unit 24-9, Fig. 2), 2) an unchanging depolarization throughout the tone burst, or 3) a gradual decrease in depolarization during the

tone burst (unit 30-1, Fig. 2). These differences may reflect variations in the location of the recording electrode with regard to both the spike-generating region and the location of inhibitory input. However, in some of these same pause cells there was evidence of inhibition manifested by a suppression of unit activity (Fig. 10) and membrane hyperpolarization to tones in the inhibitory surround (see Fig. 5) (29). The ability to detect membrane hyperpolarization to inhibitory tones but not during the pause suggests that some of the factors contributing to the pause pattern may be presynaptic.

Discharge rate as a function of tone intensity

The number of discharges evoked by tone bursts at the characteristic frequency at different intensities was determined in the four response groups in cochlear nucleus. For primarylike cells there was an increase in the number of evoked discharges with signal intensity over a 10–50 dB range (Fig. 11). In three of the cells studied the discharge rate showed a slight decrease at the highest signal intensities. For buildup cells there was no consistent rela-

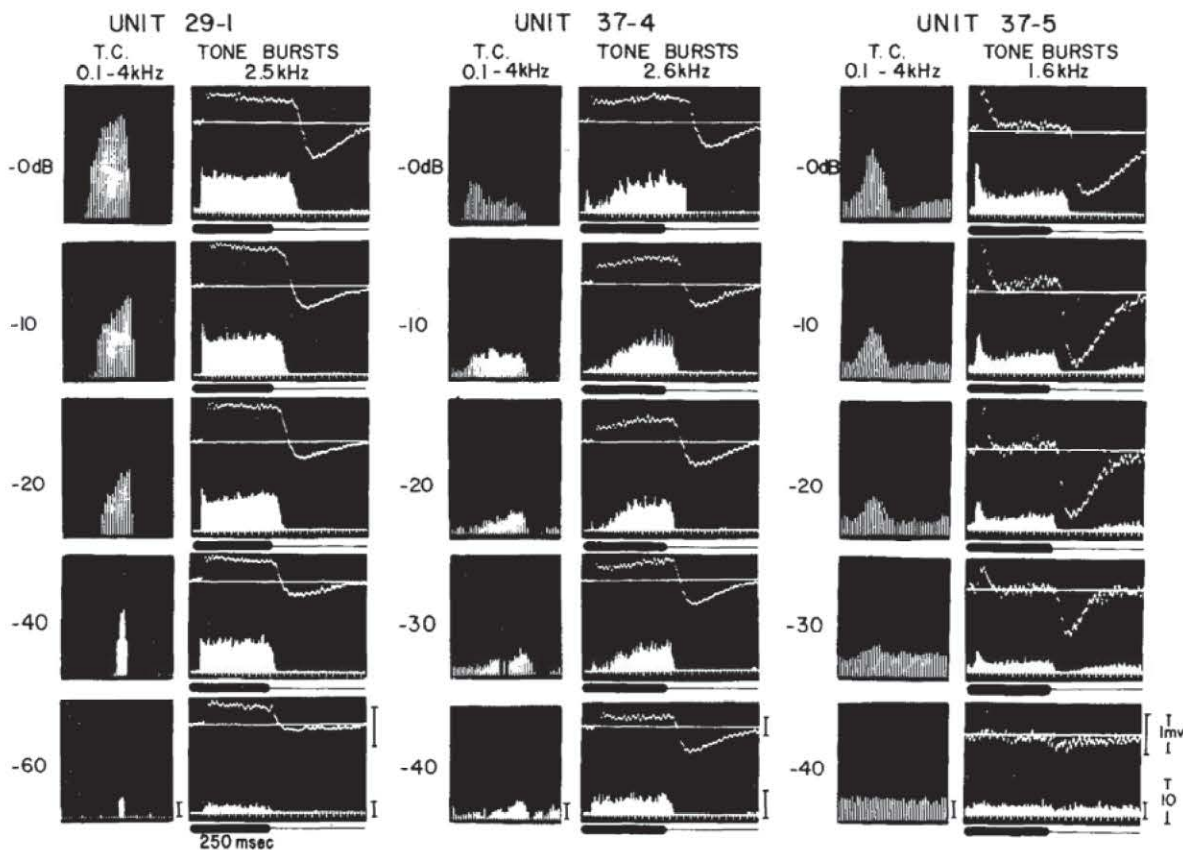


FIG. 6. Effects of intensity of the frequency-response area histogram or tuning curve (TC) and tone-burst peristimulus time histogram (PSTH) for buildup cells. Note that these units show a sustained response to low-signal intensities, which then changes to the buildup pattern as signal intensity is raised.

tion between the number of evoked discharges and signal intensity. Some units had an increase activity over a 40–50 dB intensity range (37-5, 25-6), others had no increase or even a decrease in activity (upper graphs of buildup units), and still others showed complex nonmonotonic relation (lower graphs of buildup units, 19-1, 37-4b). Onset units, in general, had little or no change in the number of discharges with changes in signal intensity. Finally, pause units had a sinusoidal nonmonotonic relation between signal intensity and discharge rate because of the lack of firing during the initial 10–60 ms of tone stimulation at intermediate intensities.

Miscellaneous tone responses

We encountered three cells in cochlear nucleus that showed only a suppression of spontaneous activity to tone bursts (6); Fig. 12 shows an example of one of these cells. The tuning curves in the upper left of the figure at -10 dB show the suppression to occur with tones ranging from 0.9 to 17 kHz. The suppression of activity is intensity dependent (right column in Fig. 11). At -0 dB, all the frequen-

cies from 1 to 10 kHz were effective but as the intensity was reduced, the inhibitory area narrowed to include only the middle-frequency range (-60 dB). A further decrease in tone intensity to -80 dB resulted in the loss of the inhibitory effects. Membrane measures show that the suppression of spontaneous activity was associated with hyperpolarization (Fig. 12, lower left).

There were eight units in cochlear nucleus whose activity did not change in response to any of our sound signals. These cells had a characteristic form of spontaneous activity marked by a regular interval between discharges. Their discharge rate, but not their regularity, increased as the microelectrode approached the cell (32).

Click responses

Although clicks were presented mainly to locate units for tone studies, there were some findings that merit comment. Unit 19-7 (Fig. 13) had recurrent membrane depolarization and discharges to clicks with a modal interval of multiples of 12 ms. The inverse of this period is equal to the period of an 80-Hz tone, which was

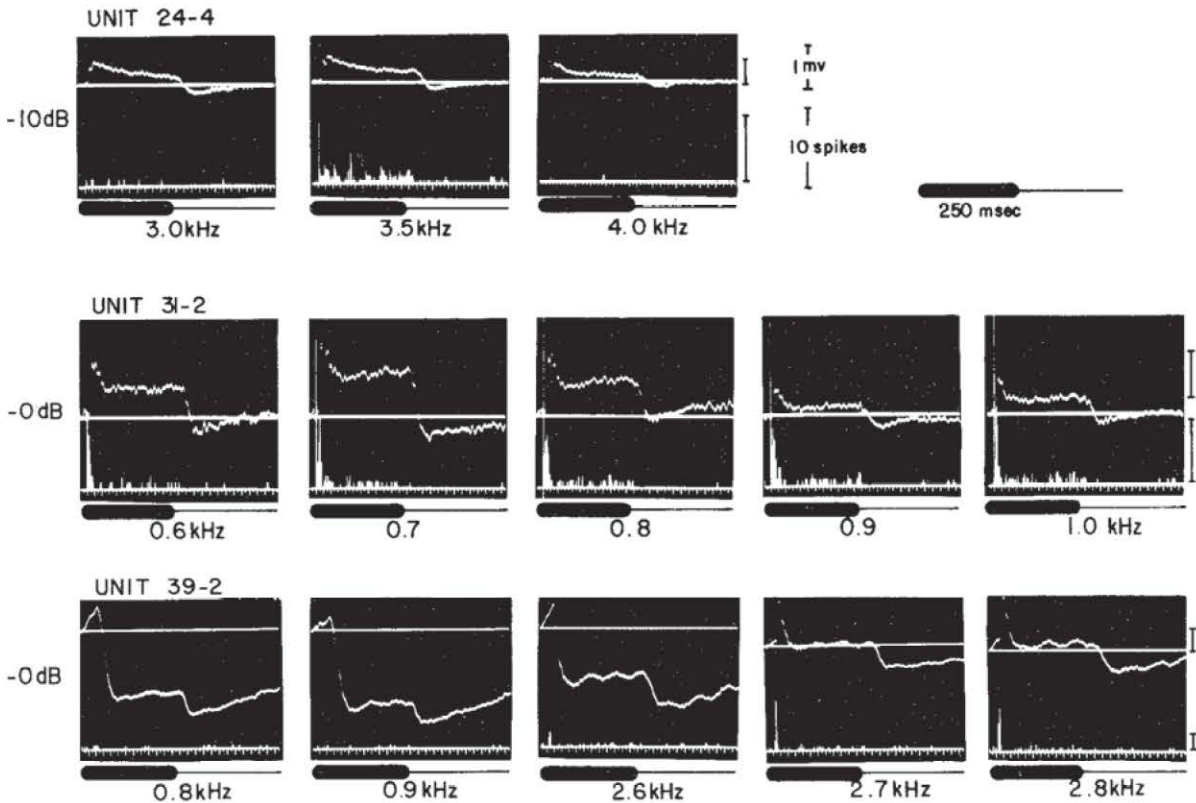


FIG. 7. Peristimulus time histograms (PSTH) as a function of stimulus frequency for three onset cells. Note that the onset pattern persists across all signal frequencies.

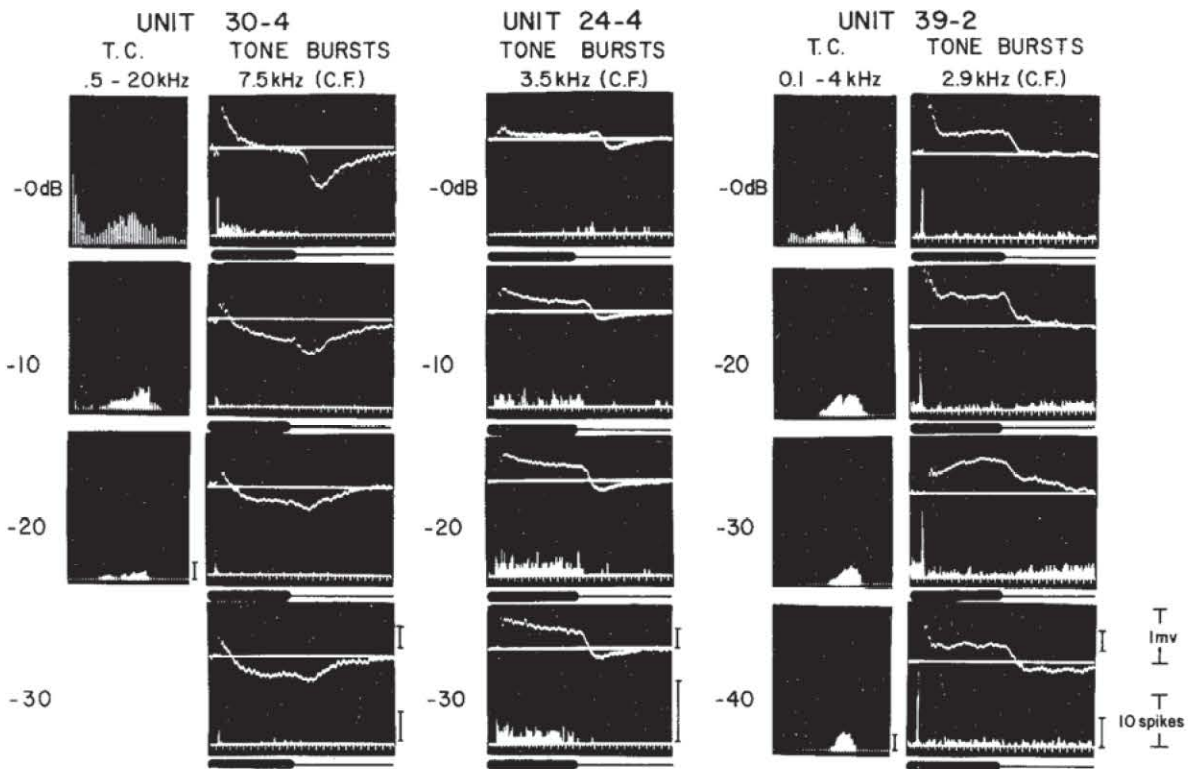


FIG. 8. Effects of intensity on the frequency-response area histogram (TC) and tone-burst peristimulus time histogram (PSTH) are shown for three onset cells. Note the onset pattern persists across all intensity levels tested.

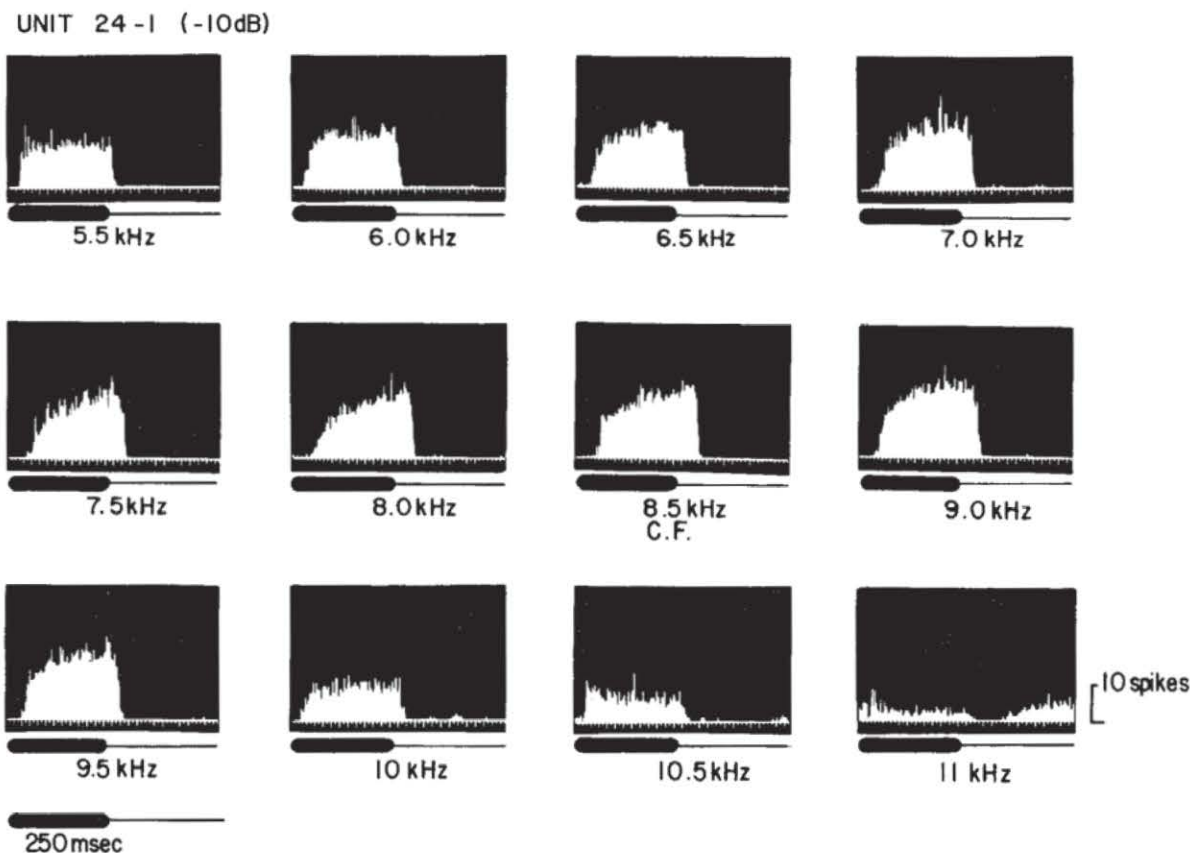


FIG. 9. Peristimulus time histogram (PSTH) as a function of signal frequency for pause cell 24-1. The characteristic frequency of this pause cell is 8.5 kHz. Note that at fringes of the response area the unit has a primarylike response. As the frequency moves toward the characteristic frequency, the latency before the unit responds lengthens and reaches 5 ms at the characteristic frequency.

this unit's characteristic frequency. Depolarization was evident in many instances without an associated cell discharge (see particularly -0, -10 dB, Fig. 13).

There were five units which responded only to clicks and not to any tone burst. It may be that the characteristic frequency was above the upper range of our capabilities (Fig. 13, unit 24-6).

There was a form of activity seen in six units in which the spike arose from the base line without a preceding synaptic event, followed by a depolarizing shift lasting 10-30 ms (unit 23-9, Fig. 13). It was possible to study this type of unit for long periods, decreasing the likelihood that the responses were due to injury. This type of activity is similar in appearance to recordings supposedly obtained from dendrites of cerebellar Purkinje cells (15).

Effects of current stimulation on tone-evoked hyperpolarization

Cathodal current (negative polarity) was used to investigate the equilibrium potentials of tone-evoked membrane hyperpolarization. Fig-

ure 14 shows the effects of cathodal current on the tone-evoked hyperpolarization of unit 25-5, a buildup cell. With -5 nA cathodal current the unit's spontaneous activity is abolished and the hyperpolarizing response is slightly diminished. With -10 nA current the hyperpolarizing response is about 50% of control value. These results suggest that the equilibrium potential of the hyperpolarization is more negative than the unit's resting potential as observed in an inhibitory postsynaptic potential or IPSP. In four other units it was possible to reduce the amplitude of the hyperpolarization evoked by tones in the inhibitory surround with cathodal current, though the equilibrium potential was never achieved. Injection of chloride ions to "turn over" the inhibitory postsynaptic potential was not attempted (5).

DISCUSSION

The change from a single discharge pattern to steady-frequency tone bursts found in eighth nerve fibers to the variety of response patterns defined in cochlear nucleus cells (6, 9, 22, 27) attests to the presence of complex synaptic

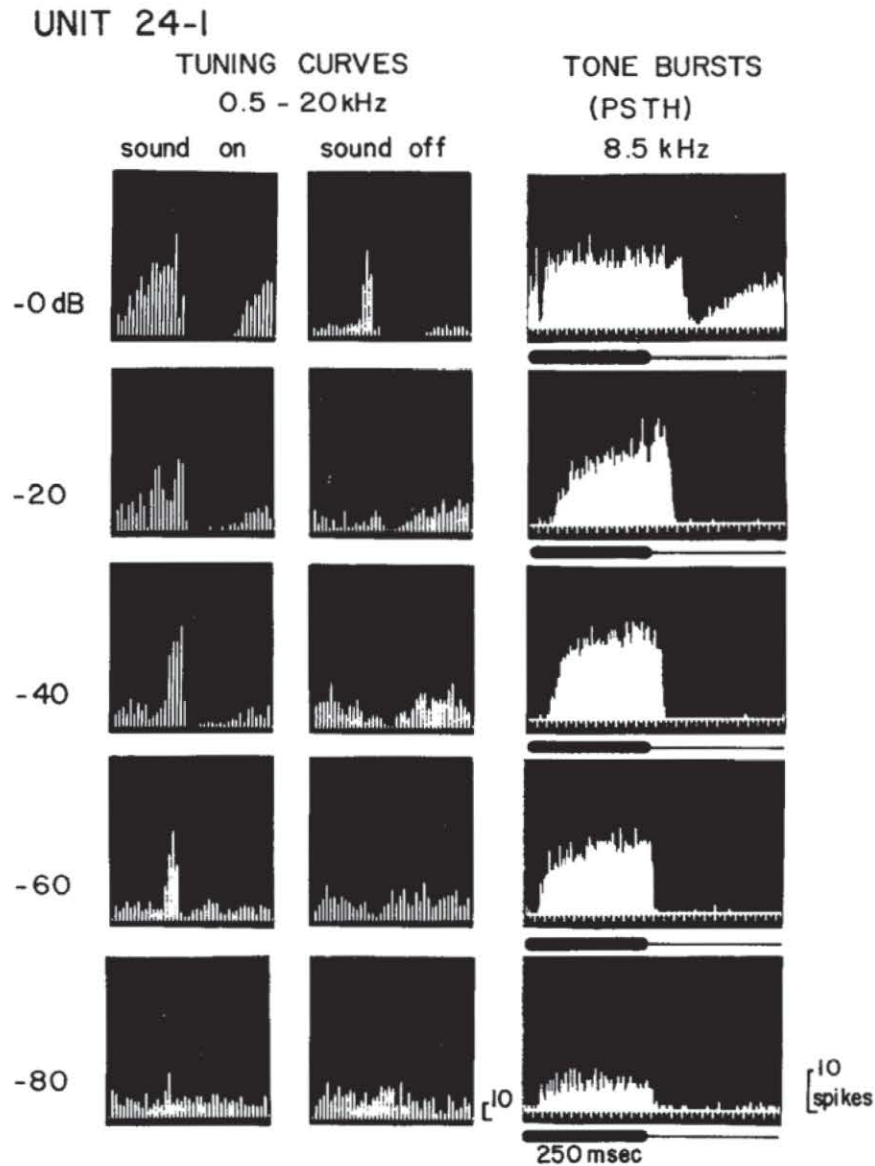


FIG. 10. Effects of intensity on the frequency-response area histogram and tone-burst peristimulus time histogram (PSTH) is shown for pause unit 24-1. In the off-count histogram, each bar represents the number of spikes occurring during the 250-ms silent period following each tone burst. The high-frequency inhibitory surround gradually diminishes with lowering of intensity. Both the off-count histogram and the PSTH at -0 dB show the off-excitation at the characteristic frequency. In the PSTH the latency gradually lengthens as signal intensity is raised. At -0 dB even the off-excitation is temporarily suppressed by the tone onset.

processing at the first central station of the auditory pathway. While granting that the classification of the response patterns is both arbitrary and dependent on signal characteristics (22), it provides a method for analyzing how neurons code the temporal aspects of acoustic signals in the auditory pathway. For instance, the occurrence of asymmetrical or unidirectional responses to frequency-modulated acoustic signals in cochlear nucleus cells can be correlated with the cells' response pattern to steady tone bursts (3).

The four response classes to steady-

frequency tone bursts distinguished in this paper include, 1) primarylike, showing a gradual adaptation in discharge rate similar to eighth nerve fibers; 2) buildup, showing a greater number of discharges evoked at the end of the 250-ms tone burst than shortly after tone onset—the buildup class corresponds to Pfeiffer's (22) pause group to 25-ms tone bursts and Evans and Nelson's response type 4 (Fig. 8 of ref 6); 3) onset, discharging principally at tone onset; and 4) pause, discharging at a prolonged latency (> 10 ms) after tone onset to tones at least 20–40 dB above threshold. The pause pat-

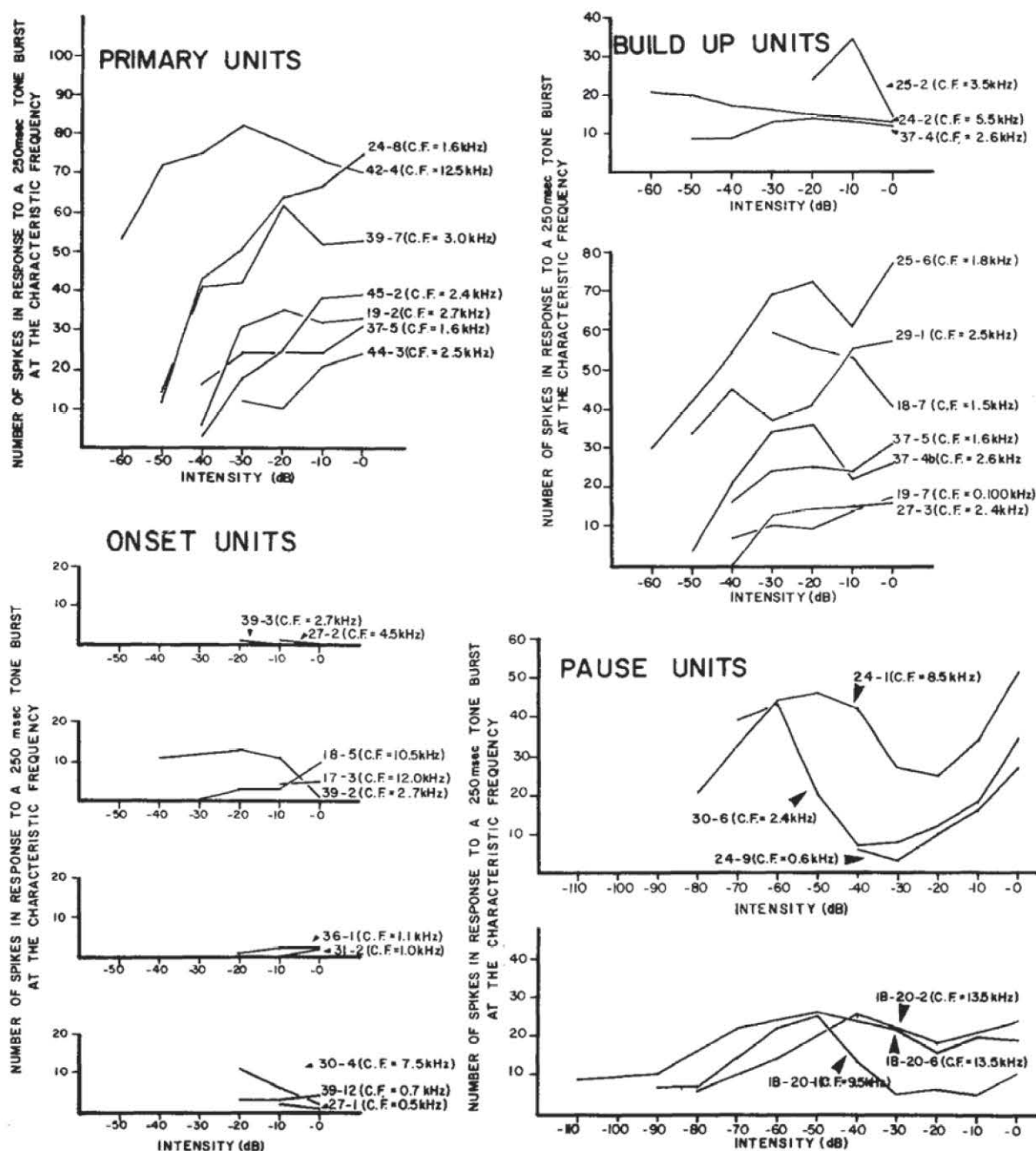


FIG. 11. Number of discharges as a function of signal intensity evoked by tones at the characteristic frequency for the four types of cochlear nucleus cells. The determination is based on the average number of discharges evoked by 40 trials of 250-ms tone bursts. Units' designation and characteristic frequency (CF) is designated to the right of each graph.

tern was not defined by Pfeiffer because his signals were within 20 dB of the unit's threshold. However pause units have been well described by Rose et al. (Fig. 16 of ref 27), and even some of Pfeiffer's histograms (Fig. 8 of ref 22) appear to be those of the pause type described in this paper.

We show in this paper that the discharge patterns for at least two of the response categories (buildup, pause) can be markedly altered by

changes both in signal frequency and signal intensity. Buildup cells can change to a sustained firing pattern with signals close to threshold, while pause cells can have a primarylike pattern when the tonal frequency is at the edges of the response area instead of at the characteristic frequency.

These results should not be too surprising if one recognizes that the response patterns reflect the temporal patterning of excitatory

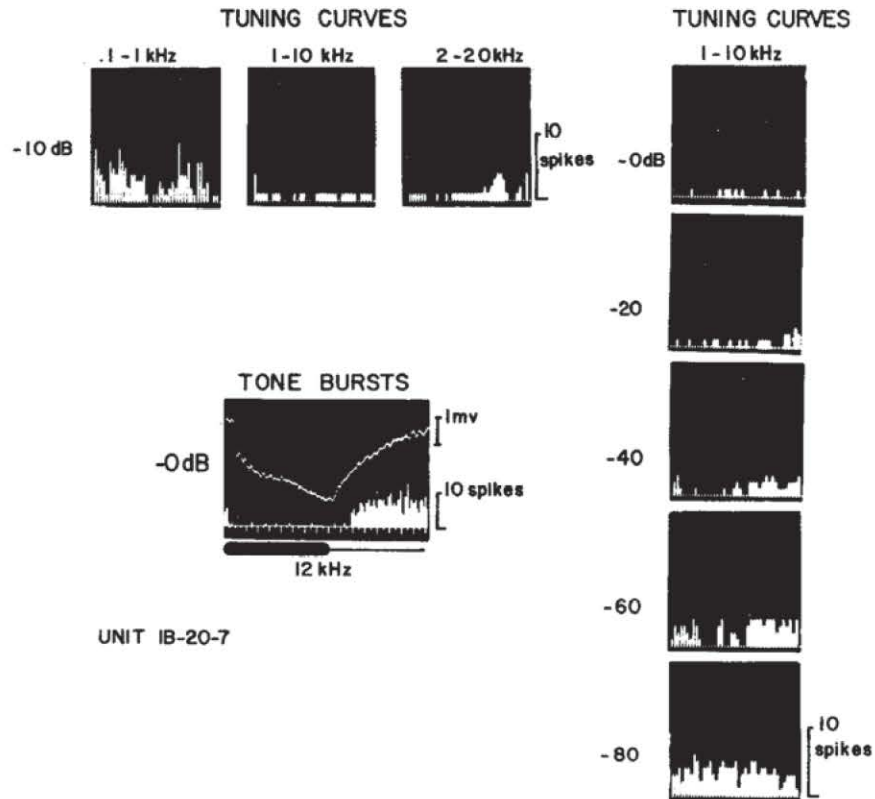


FIG. 12. An inhibitory cell. Response-area histograms (tuning curves) for three different frequency ranges are shown in upper left of photograph, an intensity series of the response-area histogram (1-10 kHz) is shown at the right, and the PSTH is at the bottom left (tone bursts). The suppression of spontaneous activity seen in the frequency-response area was accompanied by membrane hyperpolarization as illustrated in the peristimulus time histogram and average membrane potential (tone bursts).

and inhibitory events at the cell membrane. Changes in signal characteristic apparently alter the balance and temporal interaction of these synaptic drives.

Evidence of inhibitory events in cochlear nucleus cells is ample. There is membrane hyperpolarization to tones in the inhibitory surround and even hyperpolarizing shifts during portions of a steady tone burst. Such membrane hyperpolarizations during tone presentation were detected in buildup, onset, and pause cell types but not in the primarylike group. It is only with the former groups of cells that response patterns to steady tone bursts assume characteristics that are distinguished from eighth nerve activity.

Synaptic mechanisms responsible for generating each response class

Primarylike units are similar to eighth nerve fibers in their tone-burst response pattern, having an onset discharge of spikes followed by gradual adaptation, suggesting a direct and prominent input to these cells from eighth nerve fibers. The latency of activity from tone onset is brief (2-3 ms) and the membrane-

potential shifts correspond to the pattern of unit activity. There was no evidence of tone-evoked inhibitory surround manifested by suppression of spontaneous activity and membrane hyperpolarization for primarylike cochlear nucleus cells.

According to the studies of Pfeiffer (22) and Kiang et al. (12), primarylike cells are the major constituents of the anteroventral and posteroventral cochlear nucleus. Caspary (4), in his intracellular study of the kangaroo rat, was able to mark five cells showing primarylike responses with intracellular Procion yellow and defined them to be either small spherical or octopus cells.

Onset cells were primarily active only at the beginning of the tone burst. Three mechanisms are suggested to account for the onset response pattern. First, there may be feedback inhibition from an inhibitory interneuron similar to the Renshaw inhibition of anterior horn cells (25) described in the spinal cord. The initial burst of spikes from the onset cell leads to feedback inhibition limiting further discharges. Second, there may be a feedforward inhibition in which an inhibitory interneuron receives the same

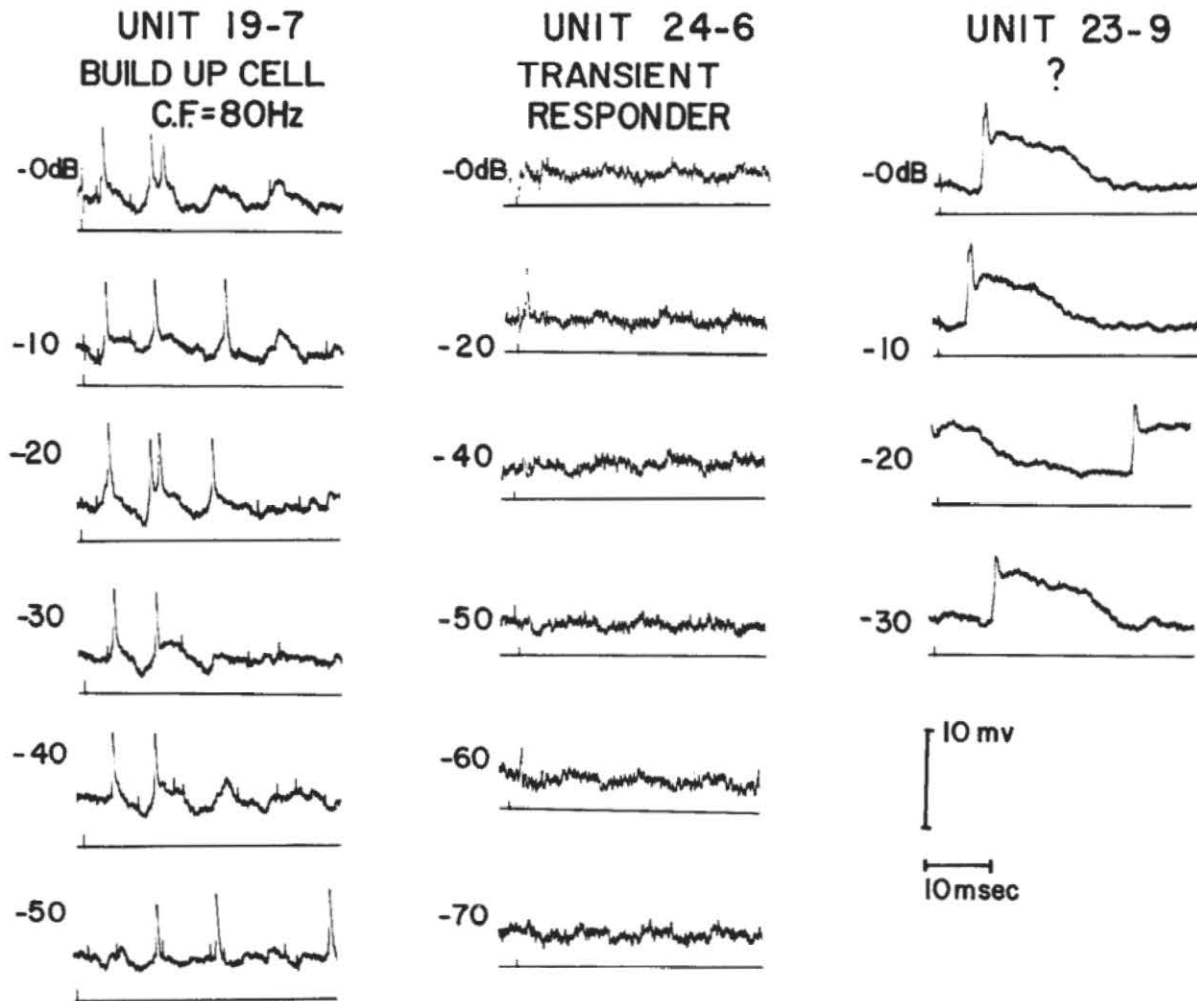


FIG. 13. Response of three cochlear nucleus units to repetitive click stimuli (25/s) as a function of intensity. The click occurs at the onset of sweeps noted by the pulse marker on the lower line of each trial. Calibrations of signal amplitude and time are given at the bottom right of the figure. See text for discussion of each unit.

input as the onset cell, but delayed in time. The onset cell is able to generate an initial burst of spikes before being inhibited. And third, the membrane of these cells may accommodate to shunt the synaptic currents. Unfortunately, membrane events recorded from onset cells do not select a particular mechanism. The membrane potentials recorded from most onset cells had an initial large-amplitude depolarization corresponding to the onset burst of spikes, followed by a lower amplitude residual sustained depolarization shift. In one instance hyperpolarization followed tone onset, suggesting that active inhibition may have been responsible for the onset pattern in this neuron.

Buildup cells have a characteristic onset burst of spikes followed by a brief suppression of activity before gradually resuming activity. In many cases the membrane potential did not correspond to the discharge pattern, but in unit 19-7 (Fig. 5) there was a prominent hyper-

polarization shortly after tone onset. Possible mechanisms for generating the buildup response include the feedback or feedforward inhibitory interneurons, similar to those proposed to onset cells except that the inhibitory influence adapts rapidly or else is inhibited itself to allow the characteristic resumption of activity in buildup units. The observation that buildup units show a sustained discharge pattern to threshold signals suggests that the inhibitory mechanisms have higher thresholds than do the excitatory mechanisms.

Pause units, characterized by their long latency of unit activity in response to tone-burst stimulation, are located primarily in the dorsal cochlear nucleus (12, 22). There is both physiological and anatomical evidence that certain cells in the dorsal cochlear nucleus receive predominantly indirect input (21, 24). Furthermore, from the anatomical work of Lorente de Nó (16) and Warr (33) it appears that at least

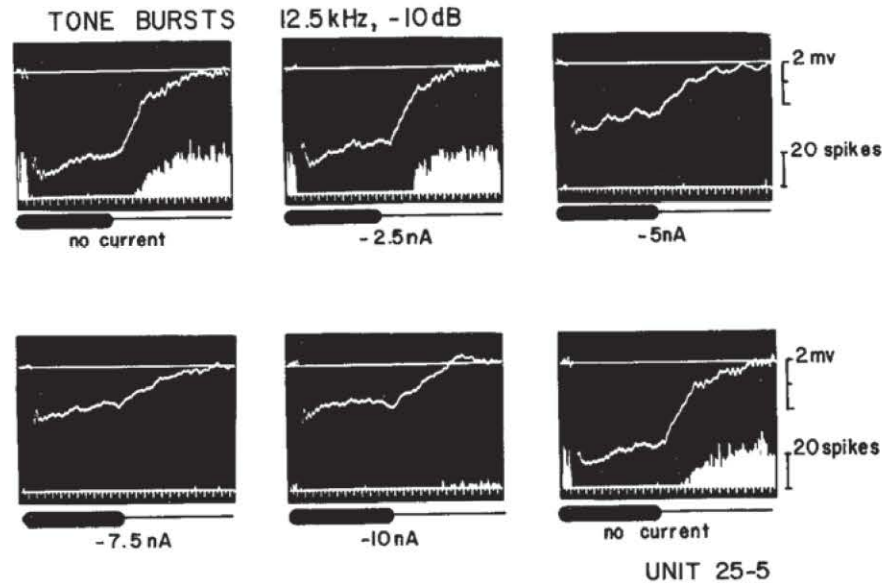


FIG. 14. Effects of cathodal current on the tone-evoked membrane hyperpolarization. The upper portion of each photograph is the averaged membrane potential and the lower portion of each photograph is the peristimulus time histogram (PSTH) of spike discharges. Forty tone bursts were used in each computation. Calibration for averaged membrane level is 2 mV and PSTH is 20 discharges. The top left and bottom right PSTHs represent controls. Cathodal current (negative polarity) was passed intracellularly in increasing strengths. At -5 nA spontaneous activity disappeared, and at -10 nA the hyperpolarization is about 50% of control value.

one source of input to the dorsal cochlear nucleus derives from ventral cochlear nucleus. Evans and Nelson (7) reported that stimulation of the ventral cochlear nucleus after extirpation of the cochlea does indeed result in short latency and prolonged suppressive effects on spontaneous activity of dorsal cochlear nucleus cells. The finding in the present experiment that the pause or delay in unit responses was not accompanied by membrane hyperpolarization and, instead, usually showed a sustained depolarization shift at tone onset suggests that the inhibition may be presynaptic. In many of these same units there was prominent tone-evoked inhibitory surrounds accompanied by membrane hyperpolarization. These results attest to the presence of complex synaptic interaction between excitatory and inhibitory events in pause units.

Inhibitory events in cochlear nucleus

There are three main inhibitory influences found in cells of cochlear nucleus: 1) tone-evoked inhibition of spontaneous activity or inhibitory surround accompanied by membrane hyperpolarization; 2) suppression of spontaneous activity and off-hyperpolarization at tone burst offset; and 3) inhibitory influences during tone bursts which account for the onset, buildup, and pause response configurations.

Tone-evoked inhibition of spontaneous activity accompanied by membrane hyperpolariza-

tion was described in cochlear nucleus by Starr and Britt (29). In the present study cathodal current was used to investigate the equilibrium potential of tone-evoked hyperpolarization. In five cells cathodal current was able to reduce the amplitude of the hyperpolarization, suggesting that the hyperpolarization had an equilibrium potential below the resting membrane potential, as is typical of most inhibitory postsynaptic potentials (5). The passage of chloride ions was not attempted in our study. Recently de Ribaupierre, Goldstein, and Yeni-Komshian (26), studying neurons in auditory cortex, were able to reverse stimulus-evoked IPSPs with hyperpolarizing current and with passive or active leakage of chloride ions from KCl-filled electrodes.

Hyperpolarization at tone offset was seen in all classes of cochlear nuclear cells (primarylike, onset, buildup, and pause). No attempt was made to study the equilibrium potential of this membrane event. From earlier studies it was shown that cells in the cochlear nucleus have a marked suppression of spontaneous activity following sound stimulation that can persist for many seconds (28) or even minutes (31). It is not known whether the change in spontaneous activity is due to alterations in afferent input since eighth nerve fibers also show a suppression of activity following tone stimulation (14) or whether the cochlear nucleus changes are mediated centrally. Starr

and Britt (29) showed that this suppression of activity at tone offset was accompanied by membrane hyperpolarization, suggesting a central neuronal mechanism for this after effect. Furthermore, they observed that discharges evoked by intracellular depolarizing currents were also suppressed at tone offset.

Functional significance of cochlear nucleus response types

Primarylike cells probably reflect the effect of direct input from eighth nerve fibers. Since they share many properties of eighth nerve fibers they may act as "relay" cells to higher auditory nuclei, as suggested by Kiang et al. (14). The primarylike cells found in the anterior ventral cochlear nucleus in turn project to the medial superior olive, which is sensitive to features of the acoustic signal relevant for sound localization (19, 20). The presence of a direct pathway from the cochlea may be advantageous for preserving the temporal cues necessary for sound localization.

Onset cells probably are important in the coding of repetitive acoustic events. Møller (18) has studied onset cells in rat cochlear nucleus which he called transient cells. Møller has shown that onset units are particularly sensitive to the time pattern of interrupted sound. Their "discharge frequency bears a precise one-to-one relationship with the stimulation as long as the silent interval between the individual sounds is longer than the minimum limit below which the particular units fails to respond." He suggests that transient or onset units play an important role in perception of the time pattern of transient sounds of low repetition rate, as is necessary for detection of periodicity pitch.

Roles for both buildup and pause cells are more difficult to hypothesize. Both of these cells tend to have prominent inhibitory surrounds or tone-evoked suppression of activity. In addition, both of these classes show changes in their temporal pattern of firing with changes in both frequency and intensity of the tone signals. These characteristics may render them sensitive to features of amplitude or frequency-modulated signals that comprise much of the acoustic signals involved in communication and in the environment.

The cochlear nucleus, the first central site in the auditory pathway, is complex in both its anatomical and its physiological characteristics. The results described in this paper reveal some of the synaptic mechanisms underlying the various response categories to steady-frequency tone bursts. In the following paper (3) the response patterns and synaptic mechanisms of

cochlear nucleus cells to FM signals will be explored.

SUMMARY

Unitary discharge patterns (peristimulus time histograms or PSTH) and synaptic events were studied with intracellular recording techniques in 164 cat cochlear nucleus cells to steady-frequency tone bursts 250 ms in duration. There were four response types defined on the basis of the shape of the discharge patterns to tones at the characteristic or best frequency.

Primarylike units resemble eighth nerve fibers and have a maximum discharge at tone onset, followed by a smooth decline to a steady level of activity. Buildup units have a transient response at tone onset, followed by a period of little or no activity before gradually increasing their discharge rate for the remainder of the tone burst. Onset units have an initial burst of spikes at the onset, with little or no activity for the remainder of the tone burst. Pause units have a long latency (10–30 ms) between tone onset and the appearance of low levels of unit activity, which then gradually increase in rate for the remainder of the tone burst.

Changes in signal frequency or intensity within the excitatory response area did not modify response patterns of primarylike and onset units, but could evoke primarylike patterns in buildup and pause units.

Inhibition manifested by suppression of spontaneous activity and membrane hyperpolarization were of three kinds: 1) in response to signals at the edges of the excitatory response area (i.e., the inhibitory surround) and detected in onset, buildup, and pause units but not in primarylike units; 2) occurring at the offset of tones in the excitatory response area and detected in all four types of cochlear nucleus cells; 3) during excitatory tone bursts in onset and buildup units associated with the periods of suppressed unit activity. Membrane hyperpolarization did not accompany the delay in unit activity after tone onset in pause units.

Inhibitory events in cochlear nucleus cells provide mechanisms for producing diversity in the temporal pattern of discharges to acoustic signals which may underly the encoding of complex features of sounds.

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