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A Physiological and Structural Study of Neuron Types in the Cochlear Nucleus. I. Intracellular Responses to Acoustic Stimulation and Current Injection

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ABSTRACT

Neurons in the cochlear nucleus differ in their discharge patterns when stimulated by tones. They also differ in their responses to depolarizing current injection in vitro. We made intracellular recordings from neurons in the cochlear nucleus of gerbils and chinchillas. The responses to tones and to depolarizing current were compared for the same neurons. Three categories of response patterns to tones were observed: chopper, primary-like, and onset. *Chopper neurons* responded with regularly spaced action potentials to stimulation with tones and to injections of depolarizing current. Their response rate rose with increasing levels of current to a maximum, which was comparable to that evoked by suprathreshold tones. These observations suggest that the regularity and maximal firing rate of these neurons are determined by voltage-dependent membrane properties. *Primary-like neurons* responded with irregularly spaced action potentials to tones. Injection of depolarizing current into these neurons produced a single action potential at current onset, which could be followed by a few irregularly spaced action potentials. The response rate showed little relation to current level. These data suggest that the membrane characteristics of primary-like neurons are different from those of chopper neurons. *Onset neurons* produced action potentials only at the beginning of the stimulus for both tones and depolarizing current, even though there was a sustained depolarization throughout the duration of the tone. The findings suggest that cochlear nucleus neurons have different membrane properties and that these properties may play a critical role in a neuron's temporal response pattern to acoustic stimulation.

Key words: auditory system, membrane properties, gerbil, chinchilla

The cochlear nucleus (CN) receives a relatively homogeneous input from cochlear nerve fibers (Kiang et al., 1965). However, CN neurons exhibit a variety of response patterns, either preserving the main features of the cochlear nerve input or transforming it. Responses of most neurons in the CN to short tone bursts in this study fall into three broad categories: primary-like, chopper, and onset (Pfeiffer, 1966; Kiang et al., 1973). Primary-like responses are characterized by a vigorous onset component, followed by an exponential decay to a stable discharge level; they resemble the responses of cochlear nerve fibers. Chopper responses consist of regularly spaced action potentials at intervals unrelated to the stimulus frequency. Onset responses have spikes locked to the start of a tone burst. Thus the CN behaves as a system that receives a homogeneous input from the cochlear nerve and responds with a heterogeneous output.

There is now considerable evidence that different response patterns are associated with specific morphological kinds of neurons. The earlier studies used extracellular recordings and their correlated microscopic observations to associate the primary-like responses with bushy cells and the chopper responses with stellate cells in the anteroventral CN (AVCN), and the onset responses with octopus cells in the posteroventral CN (PVCN) (e.g., Kiang et al., 1973; Morest et al., 1973; Kane, 1973; Brawer et al., 1974; Brawer and Morest, 1975; Godfrey et al., 1975; Bourk, 1976; Cant and Morest, 1979; Bourk et al., 1981; Ritz and Brownell, 1982; Tolbert and Morest, 1982; Tolbert et al., 1982).

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Intracellular recording and labeling of CN neurons in situ provided direct evidence that different response patterns were associated with different morphological cell types and that these morphological cell types integrate their synaptic inputs in different ways (Rhode et al., 1983a,b; Rouiller and Ryugo, 1984; Smith and Rhode, 1985, 1987, 1989; Rhode and Smith, 1986a,b). For example, primary-like discharge patterns were associated with bushy cells. They responded to tones with irregularly spaced action potentials and fast excitatory postsynaptic potentials with minimal summation. In contrast, chopper discharge patterns were commonly seen in stellate cells. A tone burst evoked regularly spaced action potentials that rode on a sustained depolarization. These results laid the background for the investigation of the role of membrane properties in shaping a cell's response to sounds.

Direct evidence that cell types in the CN have different membrane properties came from studies of brain slices (Oertel, 1983; Wu and Oertel, 1984; Oertel and Wu, 1989; Hirsch and Oertel, 1988a,b; Manis, 1990) and freshly isolated cells (Manis and Marx, 1991). Bushy cells have an outward rectification in their current-voltage relationship, i.e., they have a low membrane resistance when depolarized. Consequently, the membrane time constant is reduced when the cell is excited. This would allow a bushy cell to preserve the temporal pattern of its inputs from cochlear nerve fibers, and thus produce a primary-like response. In contrast, stellate cells have a more linear current-voltage relationship and fire with a sustained and regular discharge to depolarizing current. These features could explain the generation of a chopper response to acoustic stimuli.

In the present study, we sought to relate the membrane properties of CN neurons to the responses to acoustic stimulation. CN neurons were recorded intracellularly, and their responses to acoustic and electrical stimulation compared. Horseradish peroxidase (HRP) was iontophoretically injected into the same cells for subsequent morphological characterization. We found a correlation between a cell's response to these two kinds of stimuli. Our results indicate that three major response patterns in the CN (chopper, primary-like, and onset) reflect different membrane properties. These properties may play a role in shaping a neuron's responses to cochlear stimulation by sound. The relationship between response pattern and morphological cell type is described in a companion paper (Ostapoff et al., 1993).

METHODS

Surgical preparation

Young adult Mongolian gerbils (*Meriones unguiculatus*) and chinchillas (*Chinchilla laniger*), less than 1 year old, with clean external and middle ears served as experimental animals.

All surgical procedures conformed to the "Guide for the Humane Care and Use of Laboratory Animals," (National Institutes of Health, United States Public Health Service). Under general anesthesia (ketamine HCl, 50 mg/kg i.m., and sodium pentobarbital, 40–50 mg/kg i.p., for gerbils; ketamine HCl, 50 mg/kg i.m., and sodium pentobarbital, 70 mg/kg i.p., for chinchillas), a tracheal cannula was inserted, and a metal bar was anchored to the skull with dental acrylic and screws threaded into the skull. The head was held rigid by clamping the metal bar. Surgical anesthesia was maintained throughout the experiment.

In both the gerbil and chinchilla the CN was approached through the bulla (Frisina et al., 1982). The dorsal surface of the bulla was removed, and a small hole was made in the temporal bone lying within the perimeter of the superior semicircular canal. The hole was surrounded by a small acrylic dam and was covered with mineral oil to protect the underlying cerebellum. The electrode entered this hole and traversed the cerebellum to reach the CN. In later experiments the bulla was resealed below the vestibular canals, and the pressure in the middle ear equalized via a 20 cm polyethylene tube (PE-190) inserted into the bulla.

Acoustical stimuli

All acoustical stimuli were delivered to the right ear. After removing the pinna, a foam washer was placed against the opening of the external meatus. A sound delivery tube was pressed against this washer and sealed with Audalin (Esschem Co.). At the tip of the sound delivery tube was a probe, which was positioned about 1–2 mm from the center of the tympanum. The probe was connected to a ½ inch condenser microphone (Bruel & Kjaer). Acoustic stimuli were digitally generated and delivered through a Beyer DT-48 earphone, connected to the sound delivery tube. For each animal the level (dB SPL re: 20 μPa) and phase near the tympanum were calibrated (60–40,000 Hz in 20 Hz steps) with the probe and microphone. These calibrations were stored in a computer and used to deliver calibrated sounds. All acoustical stimuli had linear rise/fall times of 4 ms. In early experiments the mineral oil used to protect the cerebellum leaked into the middle ear and caused artificially high thresholds. For this reason we report the sound pressure level relative to neural threshold rather than absolute sound pressure level (re: 20 μPa), except where noted.

Electrodes

Electrodes were pulled on a Flaming-Brown Micropipette Puller (Sutter, P-80/PC) from borosilicate glass (OD 1.2 mm, ID 0.6 mm) with a capillary filament. They were filled with 2% or 4% HRP (Sigma type VI) in 0.5 M KCl and 0.05 M Trizma buffer (pH = 7.6 at 25°C). Each electrode was beveled (Ogden et al., 1978) until the tip resistance was 80–100 MΩ. Since chloride ions in the electrode could enter the cell and obscure inhibitory postsynaptic potentials, inhibition may be more prevalent than we observed.

Recording procedure

Recordings were made in a double-walled, sound-insulated room. A Ag-AgCl wire was used to couple the microelectrode to a high-impedance DC amplifier (Dagan, 8100). The animal ground was a Ag-AgCl pellet placed under the skin. Electrode movement and sound delivery were controlled from outside the sound-insulated chamber. The electrode was advanced by a Burleigh (PZ 550) microdrive. A LSI-11/73 computer was used to control calibration, stimulus delivery, and data collection. Intracellular recordings and stimulus parameters were stored on a multichannel FM tape recorder (EMI-SE 7000A), using a 0–2,500 Hz bandwidth. The recordings were later digitized at rates between 5,120, and 11,377 Hz.

A cell was usually impaled by passing a positive current pulse (5–40 nA, 100 ms) through the electrode. A sudden negative DC shift of at least 30 mV and the presence of synaptic potentials indicated an intracellular impalement. Impalement was often verified by passing low levels (<1

nA) of positive current to evoke action potentials. Action potentials were discriminated and timed with a 10 μs resolution. The neuron's response area and best frequency were assessed with short tone bursts (50 ms duration, presented every 100 ms). The cell was then injected with HRP by using a train of positive current pulses (100 ms on, 100 ms off) that were gradually increased to a maximum of 5 nA. This current was injected for a total of ~3 minutes in ~1 minute epochs, but it was stopped if the DC level shifted abruptly towards 0 mV. Tone bursts at the neuron's best frequency were presented in between the periods of current injection. A bridge was not used to balance the electrode resistance, and absolute current values were not recorded. However, an estimate of the relative current level was obtained from the proportional shift in the baseline voltage. Injection of sufficiently large current caused a voltage shift that saturated the amplifier. We have indicated the current levels as a percentage of the maximum current that could be delivered to each neuron without saturating the amplifier. In a few cases the current level at which the amplifier saturated was noted to be about 2 nA, but this value varied somewhat from neuron to neuron, since it depends on both the electrode and cell resistance.

Analysis

All of the analyses are based on intracellular recordings. The discharge pattern of the response to short tone bursts at the neuron's best frequency was determined from peristimulus time histograms (PSTHs: 1 ms bins). The regularity of the discharge was examined by calculating the coefficient of variation (CV) as a function of time after the onset of the tone (Bourk, 1976; Young et al., 1988; Blackburn and Sachs, 1989, 1992). We sampled the response to each repetition of the stimulus with a bin resolution of 1 or 2 ms. We then calculated the mean and standard deviation (SD) of the interspike intervals (ISIs) for the spikes in each bin over the 20–200 repetitions of the tone burst. The CV at each time was the SD divided by the mean ISI. The CV was only calculated for bins containing at least three spikes. We analyzed the first 50 ms of the response but omitted the last interval. Values of CV <0.35 indicate a high degree of regularity, whereas those >0.50 are considered irregular (Young et al., 1988; Blackburn and Sachs, 1989, 1992; Parham and Kim, 1992). Responses to current were analyzed by calculating the mean, SD, and CV of the ISIs occurring during each current pulse. These metrics were then plotted against the relative current level as defined above. In selected cases the responses to many presentations of the same stimulus were averaged to detect small potentials not readily apparent in the individual records. The synchrony of a neuron's response to a particular phase of the stimulating frequency (phase locking) was determined by calculating the vector strength (r) of the response (Goldberg and Brown, 1969).

RESULTS

Classification of discharge patterns

The temporal discharge pattern of a neuron was placed, whenever possible, into one of three categories: chopper, primary-like, or onset. These categories were based on the responses to short, suprathreshold tone bursts (~20 to 40 dB, re: threshold), delivered at or near to the neuron's best frequency. Chopper neurons fired regularly with a sustained discharge and a periodic cadence that was not directly related to the frequency of the stimulating tone (n = 11, 9 from gerbils and 2 from chinchillas; see Figs. 1, 2). Primary-like neurons fired irregularly with a sustained discharge and no evidence of a periodic cadence (see Fig. 6). Their PSTHs had a sharp onset, followed by an exponential decay to a steady discharge rate (n = 8, 3 from gerbils and 5 from chinchillas). Onset neurons responded at the beginning of a stimulus with a transient discharge and little or no sustained activity (n = 3, 1 from a gerbil and 2 from chinchillas; see Fig. 9).

Two neurons did not fit readily into these categories; these are categorized as neurons with other physiological responses.

Choppers (regularly firing neurons)

Responses to tones. The responses of ten chopper neurons are illustrated in Figures 1 and 2. The chopping pattern is usually evident from the periodic peaks in the PSTHs (left column), particularly during the initial portion of the response. The CV analysis (middle column) confirmed this periodicity, since the responses initially had CVs less than 0.35. The CV analysis allowed further discrimination into sustained (chop-S) and transient (chop-T) chopper discharge patterns. Chop-S units had small CVs (<0.35) throughout the stimulus duration (Figs. 1, 2A–C). Chop-T units showed a similar regularity initially but then became irregular (CV $>$ 0.50; Fig. 2D,E). The neuron in Figure 2D is unusual, because it began to fire regularly again near the end of the stimulus period.

The difference between the chop-S and chop-T patterns could also be seen in the intracellular traces recorded in response to single tone bursts (Figs. 1, 2, right column). For chop-S responses the action potentials were regularly spaced throughout the stimulus duration (Figs. 1, 2A–C). Chop-T responses showed regularly spaced spikes primarily during the initial portion of the tone burst, while the spikes during the later portion tended to be irregularly spaced (Fig. 2D,E). Voltage oscillations were observed during tone stimulation of some neurons (Fig. 2D,E, bottom arrow). They occurred following an action potential, and subsequent action potentials often occurred during the falling phase of an oscillation (Fig. 2D, right column, eighth action potential, top arrow). Thus, these oscillations may be artifactual and could result from overcompensation of the electrode capacitance. On the other hand, they may represent synaptic inputs from a regularly firing neuron.

Chopper neurons responded at different rates. The neurons in Figure 1A–C had the slowest chopping rates, those in Figures 1D,E and 2A had intermediate rates, and those in Figure 2B–E had the fastest rates. Chopper neurons with intermediate and fast rates generated a sustained depolarization in response to tones. This is shown in the intracellular traces in Figures 1D,E and 2 (right columns).

Responses to depolarizing current. Responses of chopper neurons to intracellular injection of depolarizing current are illustrated in Figure 3 (left and middle columns), for two chop-S (A,B) and two chop-T (C,D) neurons. Several observations can be made. First, the response rate of a given neuron increased when the current level was increased (left vs. middle column). Second, the regularity of the response increased when the current level was increased. Third, both chop-S (Fig. 3A,B) and chop-T (Fig. 3C,D) neurons responded with regularly spaced action potentials to high levels of depolarizing current. At these levels a neuron's chopping rate with electrical stimulation

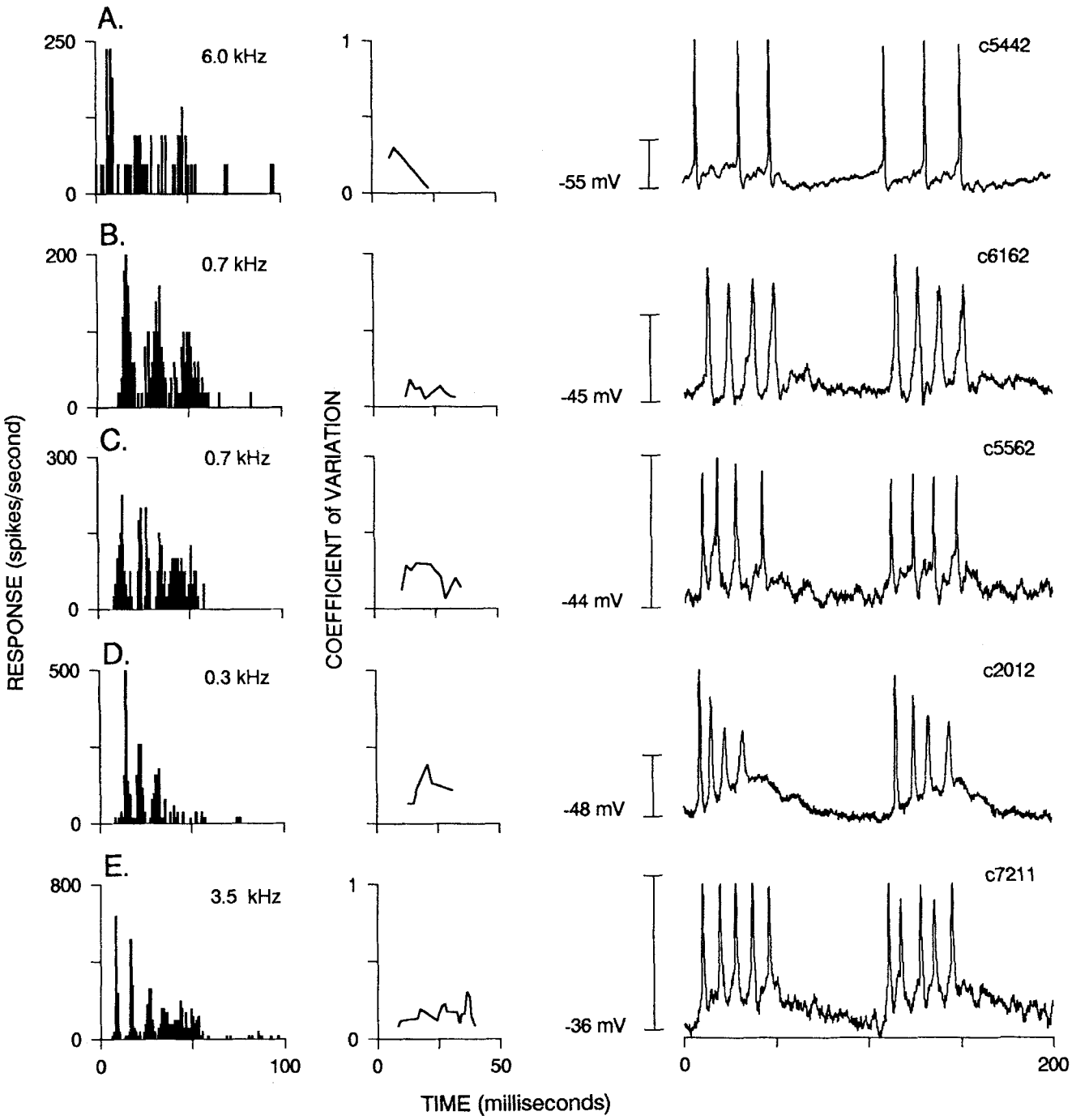


Fig. 1. Responses of five neurons with a sustained chopper (chop-S) response to tone bursts. **Left column:** Peristimulus time histograms (PSTHs): 1 ms bins, 50 ms tone bursts delivered every 100 ms. The number of stimulus repetitions used for the PSTHs, from A to E are 21, 50, 30, 50, and 50, respectively. Stimulus levels (re: threshold), for A to E, are 30, 40, 30, 30, and 20–30 dB, respectively. **Middle column:** Coefficient of variation (CV analysis) of the response (1 ms bins). Only bins with three or more intervals are plotted. **Right column:** Intracellular recordings of responses to two sequential tone bursts are shown at the same frequency as indicated in the left column. Resting potentials are given in millivolts (mV); vertical calibration bars = 20 mV. Unit numbers are posted at the right. **A–C,E:** Gerbil. **D:** Chinchilla. (Morphology of A–C and E shown in Ostapoff et al., 1994: Figs. 4, 8, 1, and 5E, respectively.)

was similar to that with acoustic stimulation (compare Fig. 3, right column with middle column).

These increases in response rate and regularity are illustrated in Figure 4, which plots mean ISI (left ordinate) and CV (right ordinate) as a function of current level for four chop-S and two chop-T neurons (Fig. 4A–D and E,F, respectively). In general, the mean ISI decreased most rapidly below approximately 50% of the maximum current level and remained at a low value above that level. Each neuron had its own asymptotic ISI. The CVs were highly regular (<0.15) at current levels above approximately 25%.

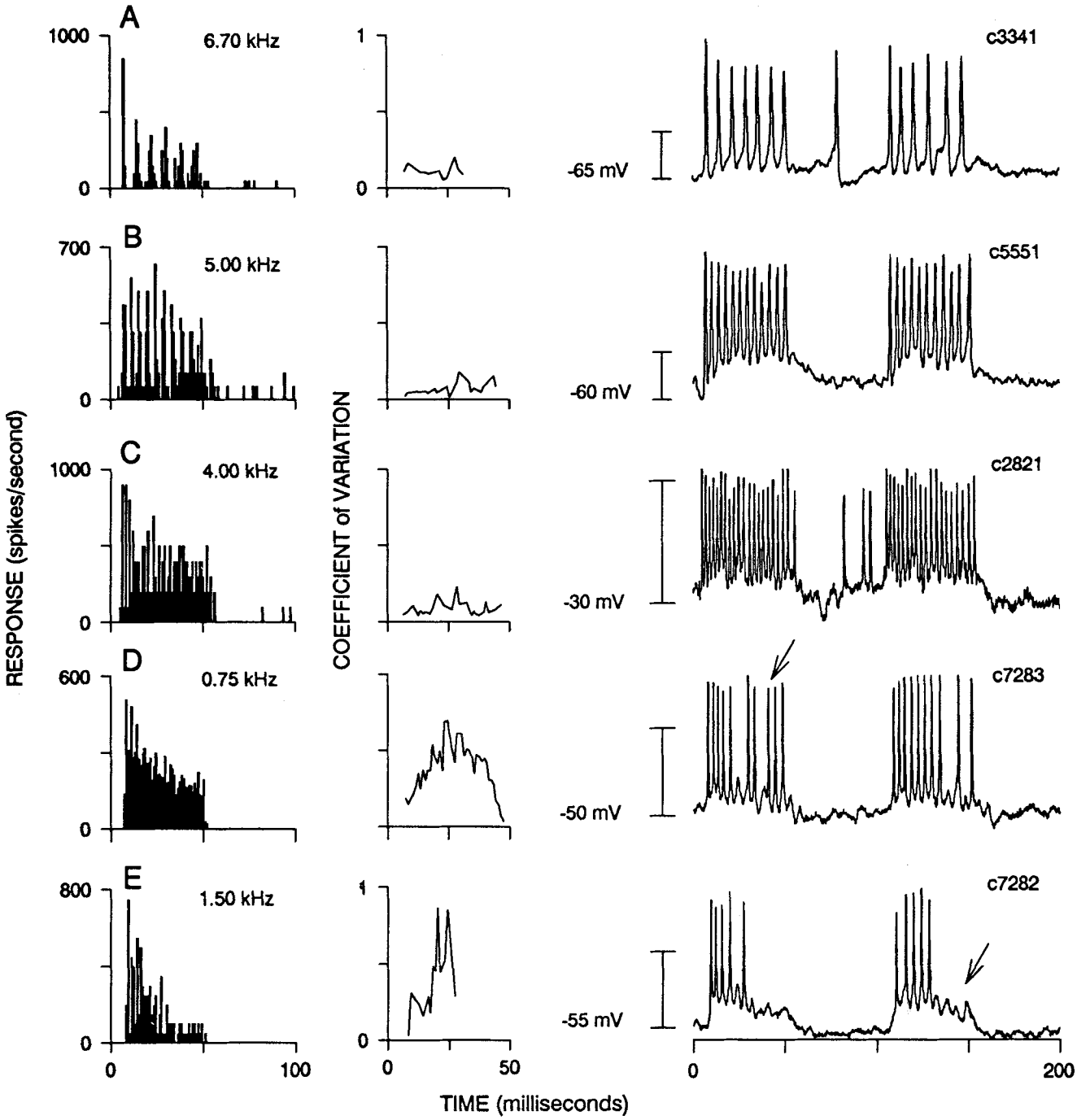


Fig. 2. Responses of three sustained (chop-S, **A–C**) and two transient choppers (chop-T, **D,E**) shown in the same format as in Figure 1. Number of stimulus repetitions for PSTHs, from A to E, are 20, 16, 10, 130, and 20, respectively. Stimulus levels (re: threshold), for A to E, are 20, 20, 40, 20–30, and 20–30 dB, respectively. A, B, D, E: Gerbil. C: Chinchilla. Arrow, voltage oscillations (see text for explanation). (Morphology of A and B shown in Ostapoff et al., 1994: Figs. 5B and 7, respectively.)

Relationship between the responses to tones and current. Figure 5 illustrates the relationship between electrical and acoustical stimulation by plotting the mean ISI (±SD) of responses to tone bursts against the same metrics of response to maximal current level (~2 nA) for the six neurons in Figure 4. Neurons with short ISIs in response to tones also had short ISIs at maximal current level. Similarly, those with long ISIs in response to tones also had long ISIs with maximal current. The mean ISIs of the responses to tones were slightly smaller than those evoked by the maximum current levels for five of the six neurons.

Primary-like (irregularly firing neurons)

Responses to tones. Figure 6 illustrates examples of irregular discharge patterns from six neurons (same format as Figs. 1 and 2). The PSTHs were classified as primary-like (Figs. 6A–E, left column) or primary-like-with-notch (Fig.

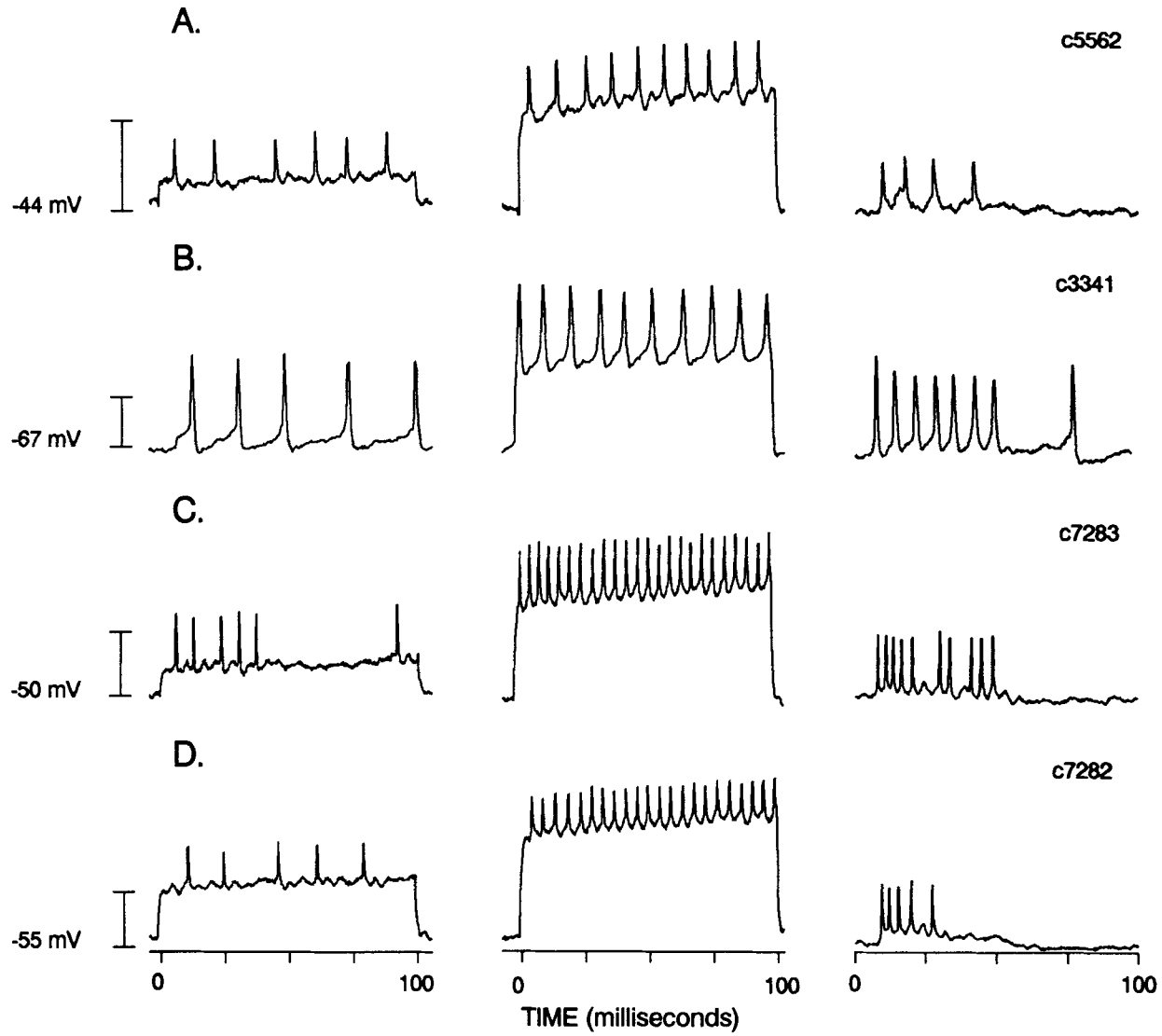


Fig. 3. Responses of two chop-S (**A, B**) and two chop-T (**C, D**) neurons to positive current injections (**left and middle columns**) and to single tone bursts (**right column,** taken from Figs. 1C and 2A,D,E). Current levels in the left column for A–C are less than 8% of maximum and for D, 20% of maximum; those in the middle column are between 24% (A) and 45% (D) of maximum. Chop-S neurons are the same as those in Figures 1C and 2A. Chop-T neurons are the same as those in Figure 2D and E. Resting potentials in mV; vertical bar = 30 mV.

6F). The primary-like-with-notch responses had a 1–2 ms pause following the initial spike. The CVs of these units (middle column) were relatively large (CV > 0.50) and could have large fluctuations. None had the regularity observed in chopper neurons. The irregularity of primary-like responses is also seen in the intracellular traces depicting responses to two tone bursts (right column). These intracellular records often had rapid fluctuations of variable amplitude, a feature that made it difficult to distinguish synaptic potentials from action potentials. In such cases the recordings were triggered on the larger potentials. Unlike most choppers, none of these neurons had a sustained depolarization during the tone burst.

Responses to current. Neurons with primary-like responses produced an onset spike when injected with depolarizing current (n = 5). The responses to current from the first four neurons in Figure 6 are shown in Figure 7. Starting from the left, responses to three current pulses at progressively higher levels are shown. In contrast to choppers, these neurons did not respond robustly to current. In all cases shown, an initial spike was evoked at the highest current levels. The initial spike was often followed by a few, irregularly timed action potentials (4 of 5). Three of these four neurons also had spontaneous activity (Fig. 7B,D).

Unlike choppers, which showed a monotonic decrease in ISI as a function of current level (Fig. 4), the primary-like neurons usually did not show such a relationship. Figure 8A and B shows the mean ISI and CV for the neurons in Figure 7B and C, respectively. With increasing current levels the ISIs of the neuron in Figure 8A tended to increase, while those of the other neuron tended to decrease (Fig. 8B). However, both neurons showed erratic fluctuations in ISI and CV. The responses could be highly irregular (CV > 1.0).

Onset neurons

Responses to tones. One neuron from a gerbil and two from the chinchilla responded to tones with an onset discharge pattern (Fig. 9, left column). The neurons of Figure 9A and B responded to each tone with a single,

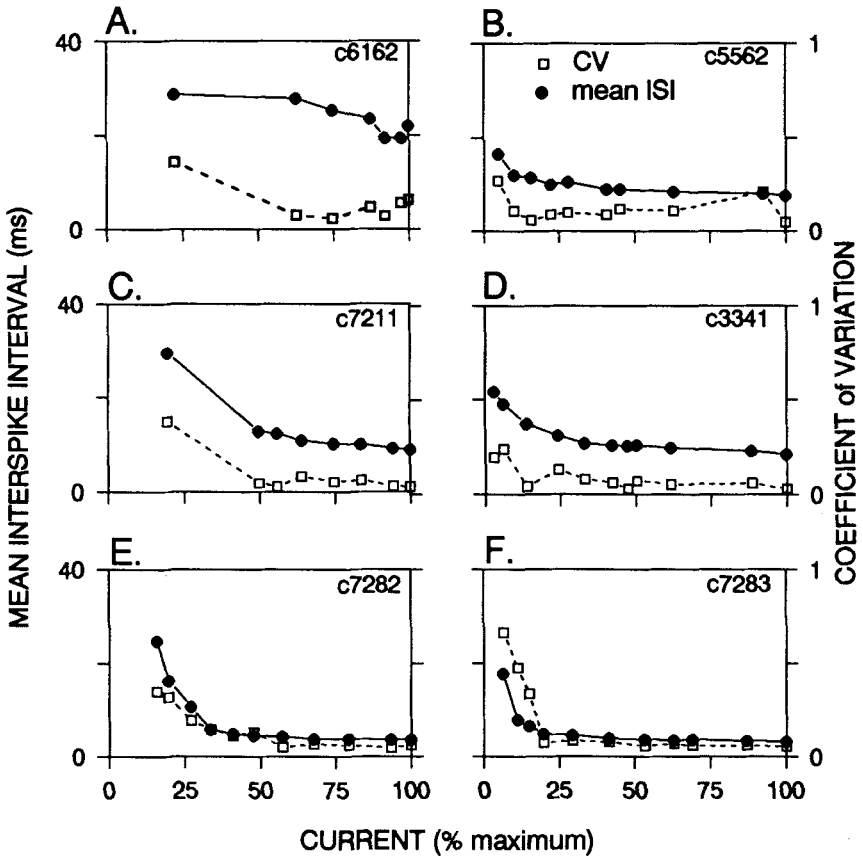


Fig. 4. Responses of four chop-S (**A–D**) and two chop-T (**E, F**) neurons to different levels of depolarizing current. Plotted are the mean interspike intervals (ISI; left ordinate, solid circles) and CV (right ordinate, open squares) vs. relative amplitude of current. Chop-S and chop-T responses for B and D–F are from the same neurons shown in Figure 3. A and C are from the chop-S responses in Figure 1B and E, respectively. Responses to current for these two neurons are not shown.

tightly locked action potential (SD of first spike latency < 0.5 ms). In contrast, the neuron of Figure 9C produced loosely locked action potentials (SD = 3 ms), which resulted in a diminution of the peak in the PSTH and the averaged intracellular record (middle column). This neuron occasionally discharged a second action potential (right column). All three units showed a sustained depolarization for the duration of the tone burst. The sustained depolarization could be observed in some responses to individual tone bursts (right column), but it was more clearly seen in the averaged records (middle column).

Responses to current. Figure 10A and B shows the responses to increasing levels of current from the units in Figure 9B and C, respectively. Current levels in the left column are subthreshold. At threshold a single action potential was evoked at the onset of the depolarizing current pulse (middle column). Further increases in current did not produce additional action potentials (right column).

Other response features

In this section we describe evidence for inhibitory events that may shape the response profile of choppers. We also present the response properties to tone and current of a neuron that displayed precise phase locking.

Inhibitory influences. The possibility that inhibitory processes play a role in modulating the response of choppers is suggested by the hyperpolarizing potentials observed in a chop-S and a chop-T neuron in response to tones above their best frequencies.

Figure 11 illustrates recordings from a chop-T neuron (same cell as in Fig. 2D). The averaged response (left column) and responses to two sequential tone bursts (right column) are shown at several frequencies. At the highest frequencies tested (2.5 and 2.25 kHz; Fig. 11A,B), the stimulus evoked only a sustained hyperpolarization. The averaged response to 2.0 kHz was a transient depolarization, followed by hyperpolarization (Fig. 11C). The response to individual tone bursts (right column) had synaptic potentials suggestive of inhibitory postsynaptic potentials (IPSPs) (arrows). At progressively lower frequencies the initial excitation and accompanying sustained depolarizations became larger and longer (Fig. 11D–F). This effect would obscure any later hyperpolarizations, if present. The presence of hyperpolarization, even when the net response is excitatory, is suggested by the decay of sustained depolarizations, accompanied by presumptive IPSPs during the later segments of the stimulus (e.g., Fig. 11D). Transient chopping was evident at frequencies near the neuron's best frequency (Fig. 11E,F).

Phase locking. One neuron was remarkable for its phase locking. This neuron was located in the posteroventral CN; while its morphological type was uncertain, it was not a bushy cell (Ostapoff et al., 1994). Its phase locking was

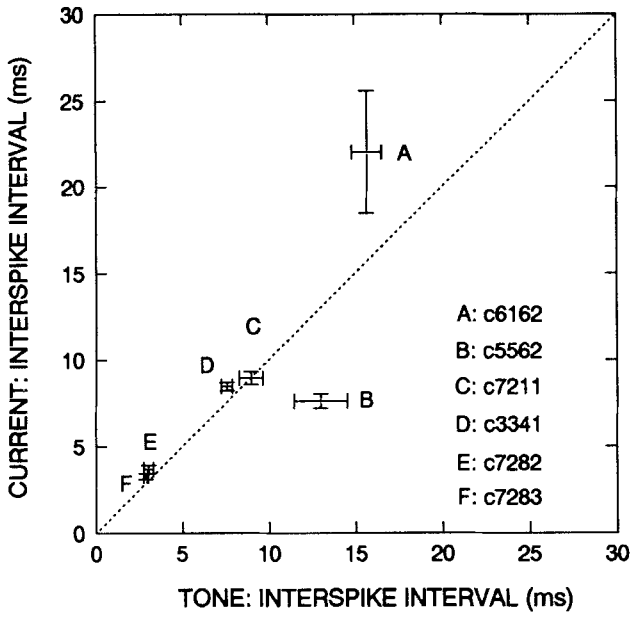


Fig. 5. Relationship between the mean ISI in response to tones and the mean ISI in response to positive current injections for four chop-S (A–D) and two chop-T (E, F) neurons. ISIs (± SD, vertical error bars) with current are from the responses to maximum current shown in Figure 4A–F. ISIs (± SD, horizontal error bars) with tones are calculated from the corresponding responses shown in Figures 1 and 2, left columns. Dots indicate the line of equality.

highly effective and very precise. Thus, to a 100 Hz tone it fired an action potential for each cycle of the tone at nearly the same phase. Figure 12 shows its responses to stimuli at several frequencies (100–700 Hz), all at the same intensity (70 dB SPL). The first column shows the PSTH, the second column the period histogram, the third column the averaged intracellular record, and the fourth column the intracellular responses to two sequential tone bursts.

As stimulus frequency increased, the discharge changed from a sustained to a more transient pattern (Fig. 12, first column). Furthermore, the precision and effectiveness of phase locking systematically decreased (second column, r: 0.93–0.49); the amplitude of the sustained depolarization increased (third and fourth columns) with increasing stimulus frequency. At 100 Hz a sustained depolarization was not evident; instead, the membrane hyperpolarized after each spike.

Phase-locked excitatory postsynaptic potentials (EPSPs) are seen at 300 Hz (Fig. 12, fourth column, arrows). These are more clearly seen in Figure 13, which illustrates the responses of this neuron to a lower stimulus intensity (60 dB SPL). The single EPSP at 100 Hz (Fig. 13, arrow) is quite broad. At 200 and 300 Hz these phase-locked EPSPs are prominent and accompanied by a sustained depolarization. At higher frequencies only a sustained depolarization is evident. As in the response at 70 dB (Fig. 12), the discharge pattern changed from sustained to transient with increasing stimulus frequency.

Figure 14 depicts this neuron's response to increasing levels of depolarizing current. At each of the levels shown there was an onset spike, followed by a few (one to three) irregularly spaced spikes. However, onset spikes were not always present, and the driven activity at all current levels was low and variable.

DISCUSSION

The present study relates the responses from acoustic stimulation of different cell types in the CN to their intrinsic membrane properties. The findings have implications for understanding auditory signal processing.

We observed three basic patterns of response to tone bursts in the CN. Choppers had regularly spaced action potentials, which were associated with a sustained depolarization. Primary-like neurons had irregularly spaced, rapid fluctuations in potential, which varied in amplitude and had little, if any summated depolarization. Onset neurons produced only an initial action potential or two, accompanied by a sustained depolarization.

Each of these response patterns to tones was associated with a specific discharge pattern for stimulation with depolarizing current. Choppers responded to depolarizing current with regularly spaced action potentials and discharge rates that increased with current level. There was a positive relationship between a chopper's discharge rate in response to tones and to current. Primary-like neurons responded to current with an initial action potential, which could be followed by a few irregularly spaced action potentials. There was little, if any relationship between current level and the discharge rate or between the discharge rate for tones and that for current. Onset neurons produced only a single action potential in response to current pulses or tones.

Chopper neurons

Chopper neurons corresponded to several different morphological cell types (see Ostapoff et al., 1994). These neurons had similar firing rates for tones and for current (Fig. 5). The deviations from equality may result from several factors. In some cases the response rates for tones may not have been maximal, because the optimal acoustical stimulus was not determined during the short time for which a cell could be studied. However, the mean dynamic range for choppers is reported to be 29 dB (Rhode and Smith, 1986a) and our tone-current comparisons used stimulus levels near this range (20–40 dB, re: threshold). In other cases the response rates for current may not have been maximal, because the location and quality of impalement limited its efficiency in evoking spikes. Thus it is likely that the similarity between the discharge rate for tones and for current is even closer than observed. This similarity no doubt reflects the fact that the membrane properties of a chopper limit its maximal response.

Chopper neurons had considerable variation in their maximal discharge rates (Fig. 4). Differences in maximal chopping rates between neurons may arise because different neurons have ionic channels with different kinetics. Manis and Marx (1991) described isolated CN neurons that fired regularly in response to depolarizing current. These neurons had a slow outward conductance that controlled the duration of the hyperpolarization following each action potential. The time constant for this conductance varied over a wide range (6.2–18.0 ms). Since a hyperpolarization following the action potential influences the ISI, such a variation in the time constant of this conductance may explain the wide range of maximal chopping rates observed here and in other studies (Bourk, 1976; Young et al., 1988; Blackburn and Sachs, 1989, 1992; Rhode and Smith, 1986a).

The sustained depolarization, observed in most chopper neurons here and by others (Rhode et al., 1983b; Rhode and

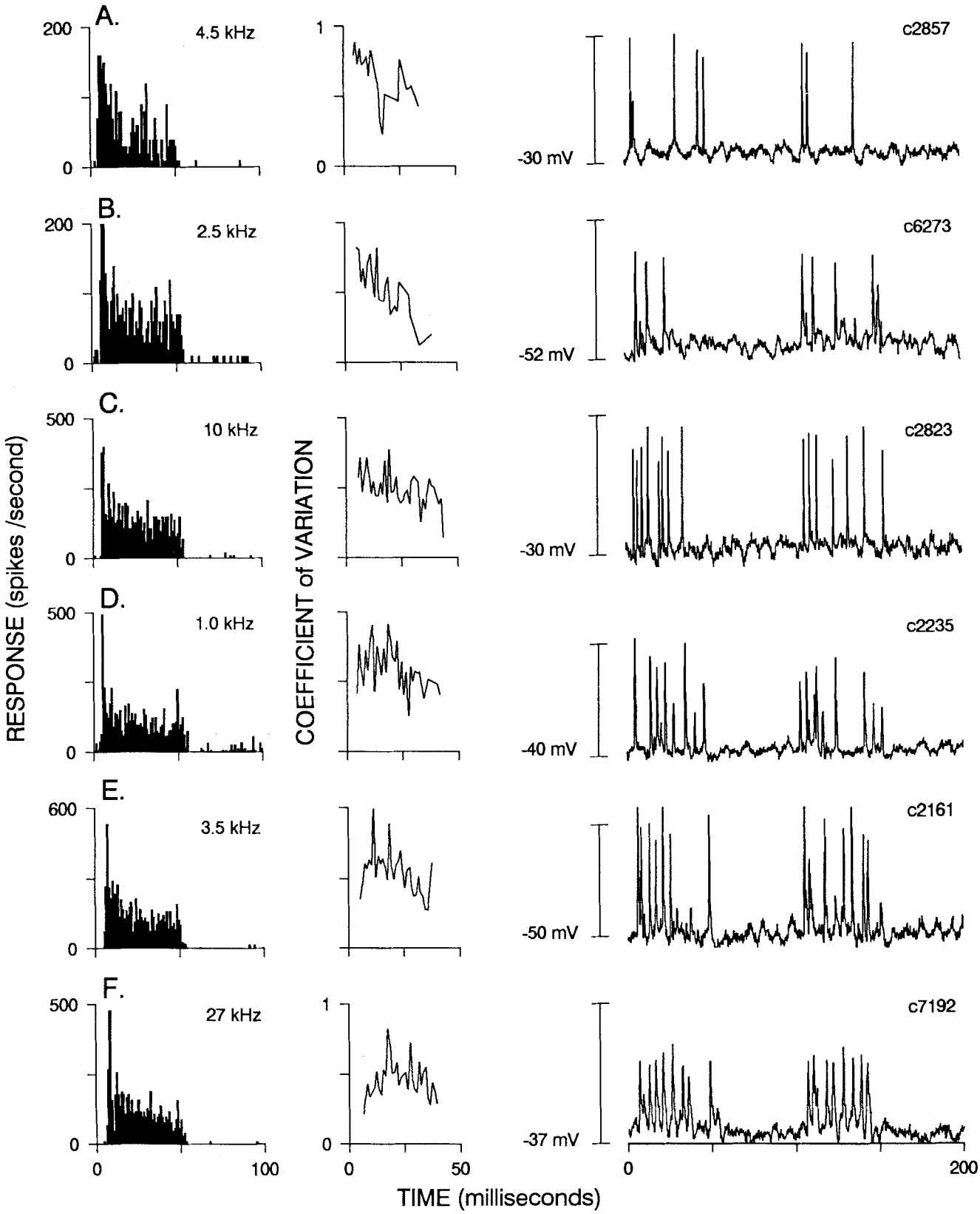


Fig. 6. Primary-like (**A–E**) and primary-like-with-notch (**F**) responses to tone bursts at the frequencies indicated (same format as in Fig. 1). Repetitions on which PSTHs are based, from A to F, are 50, 100, 50, 20, 115, and 100, respectively. Stimulus levels (re: threshold), for A–F, are 30, 30, 40, >50, 40, and 30 dB, respectively. A, C, D and E: Chinchilla. B and F: Gerbil. Calibration bars = 20 mV. (Morphology of E shown in Ostapoff et al., 1994: Fig. 10.)

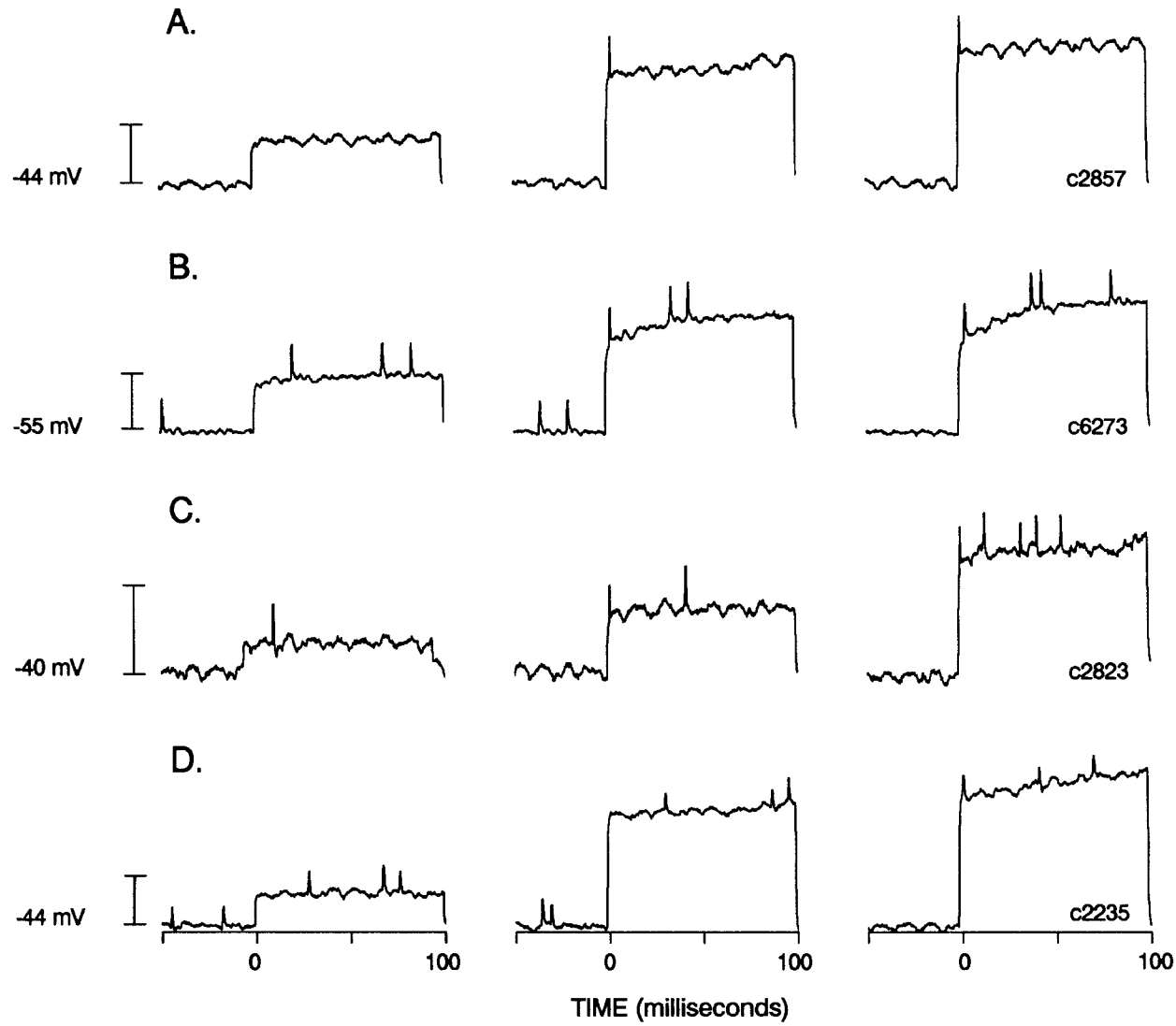


Fig. 7. Primary-like responses (**A–D**) to increasing levels of depolarizing current (left to right columns). **Left column:** Subthreshold current levels. **Middle column:** Near-threshold levels (16% of maximum current for C to 50% for D). **Right column:** Suprathreshold levels. The higher stimulus levels evoked action potentials at the onset of the pulse. Same neurons as in Figure 6A–D. Calibration bars = 30 mV.

Smith, 1986a; Smith and Rhode, 1989), supports the hypothesis that the membrane of these neurons has a long time constant. This would favor the summation of synaptic potentials. Recordings from brain slices (Oertel, 1983; Wu and Oertel, 1984) and isolated neurons (Manis and Marx, 1991) indicate that regularly firing cells in the CN have long membrane time constants. Models of chopper cells have used a long membrane time constant to produce a regular discharge (Arle and Kim, 1991; Banks and Sachs, 1991). It is possible that the dendrites of these cells receive large numbers of cochlear nerve terminals—a feature that could contribute to a sustained depolarization.

Primary-like neurons

The primary-like response to acoustic stimulation had rapidly fluctuating potentials with varying amplitudes that were irregularly spaced. The presumed "subthreshold" potentials of varying amplitude may reflect synaptic inputs at varying distances from the electrode, i.e., axosomatic versus axodendritic. It is also possible that they reflect single and summed synaptic events evoked by cochlear fiber inputs (Smith and Rhode, 1987; Smith et al., 1993). There was no evidence of a sustained depolarization during stimulation with tones. Their response properties are comparable to those described for primary-like units and bushy cells in the cat CN (Rhode et al., 1983b; Rhode and Smith, 1986a; Smith and Rhode, 1987) and in the present study (Ostapoff et al., 1994).

In the present study, primary-like units responded to depolarizing current with an action potential at the onset of the current step. The first spike could be followed by irregular firing, especially if the neuron showed spontaneous activity. There was little, if any, relationship between the current level and the average rate of the irregular discharge (see Figs. 7, 8). This behavior is consistent with that of bushy cells observed in CN slices from the mouse

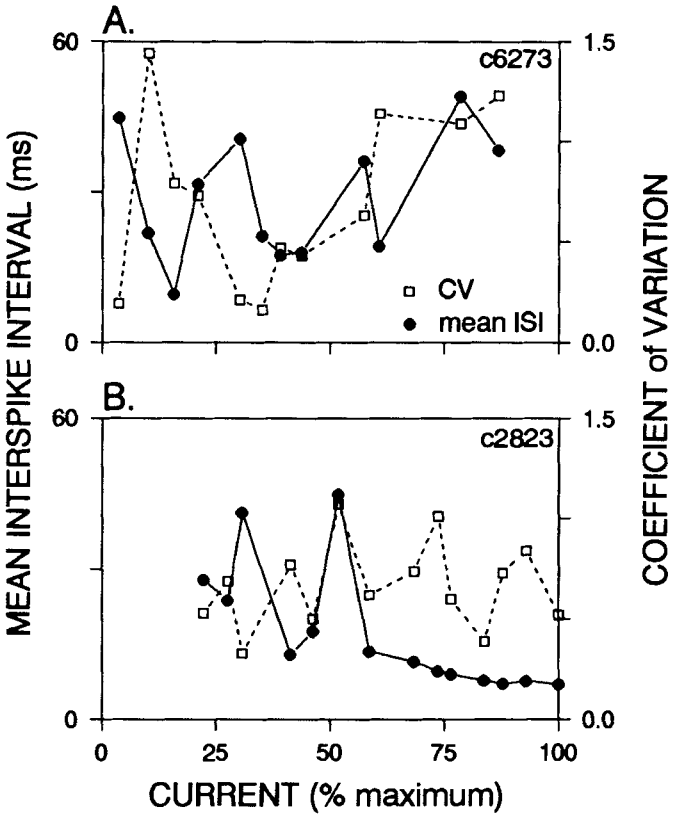


Fig. 8. Responses of two primary-like units **(A, B)** with sustained discharge to depolarizing current (same neurons from Fig. 7B,C). The format is the same as in Figure 4, with the mean interspike intervals (ISI) (left ordinate and solid circles) and CV (right ordinate and open squares) plotted vs. relative amplitude of current. The scales for both ordinates are different than in Figure 4.

(Oertel, 1983; Wu and Oertel, 1984) and in presumed bushy cells acutely isolated from the guinea pig CN (Manis and Marx, 1991). Under such conditions these cells did not support sustained activity with depolarizing current. The input resistance of these cells decreased during depolarization, a feature that does not favor the repetitive generation of spikes. In the present case some primary-like cells responded to current with action potentials subsequent to the initial spikes (Fig. 7B–D). These subsequent spikes may result from the spontaneous activity of cochlear nerve inputs observed in situ, but not in vitro. This spontaneous activity may evoke spikes from a primary-like cell, even when its membrane resistance is reduced by intracellular current injection. The presence of irregular spike discharges during the low-level current step (Fig. 7B,D, left column), in the absence of spikes at the onset of the current, supports this explanation. The response features of primary-like neurons have been associated with a low-threshold potassium conductance, which reduces the membrane time constant and thereby keeps synaptic events brief (Manis and Marx, 1991). Blocking this potassium conductance with 4-aminopyridine in principal cells of the medial nucleus of the trapezoid body caused rectified current-voltage relationships to become more linear (Banks and Smith, 1992). Depolarizing current that previously evoked an action potential only at the onset could now evoke regular firing. Thus, as in the medial nucleus of the trapezoid body, a low-threshold potassium conductance may play a role in preserving the primary response patterns in the CN.

Onset neurons

The onset neurons responded to tone bursts with one or two action potentials at the onset of each stimulus and with a sustained depolarization that lasted for the duration of the stimulus. Two of the neurons were located in the posteroventral CN (Ostapoff et al., 1994) and had an initial action potential that was precisely timed. A third neuron was located in the anteroventral CN, and it had a variable onset latency. Romand (1978) also recorded onset responses with sustained depolarizations from neurons located in both the anteroventral CN and posteroventral CN. Onset neurons with a single, precisely timed action potential have been described as ideal onset units, O_I (Godfrey et al., 1975; Adams, 1976; Rhode and Smith, 1986a). In response to current, one of our O_I units had a single action potential at the beginning of the pulse (Fig. 10). So did the unit with a variable onset pattern.

The sustained depolarization in response to tones suggests that the failure of these neurons to maintain a discharge is not caused by inhibition or lack of an excitatory input. This interpretation is supported by the observation that depolarizing current also evoked only an onset action potential. Such stimulation would not be expected to activate inhibitory inputs, except via a feedback loop, which might involve a significant delay.

Depolarization block is one possible mechanism, intrinsic to the onset neuron, that could produce an onset response. First observed in the squid giant axon, depolarization block can result from inactivation of a sodium conductance (Hodgkin and Huxley, 1952). Granit and Phillips (1956) pointed out that this mechanism could explain the suppression of activity seen in cerebellar Purkinje cells. Ritz and Brownell (1982) proposed depolarization block as the basis for the onset response of neurons in the posteroventral CN. An alternative mechanism might involve a potassium conductance sensitive to 4-aminopyridine, such as that proposed for bushy cells (Manis and Marx, 1991) and neurons in the medial nucleus of the trapezoid body (Banks and Smith, 1992).

While the depolarization block mechanism may explain ideal onset responses (O_I) to acoustic stimulation, it alone does not explain some other types of onset responses observed in the CN. These other onset categories (less than ideal onset, O_L, and onset chop, O_C) are actually sustained responses, but the first spike or first few spikes are tightly locked to the onset of the tone burst. The early, well-timed spikes produce large peaks in the PSTH. The later, sustained portion can have rates as high as 200/s or more (Rhode et al., 1983b; Smith and Rhode, 1989). Such a sustained discharge rate is not consistent with a depolarization block initiated after the first action potential.

The O_I responses have been associated with octopus cells on the basis of extracellular (Godfrey et al., 1975) and intracellular data (Rouiller and Ryugo, 1984; see also Ostapoff et al., 1994). However, other kinds of onset responses have also been associated with octopus cells. Godfrey et al. (1975) reported that O_L and O_I responses were found in the octopus cell area of PVCN. Rhode et al. (1983b) described an octopus cell with an O_L response to tones, while Joris et al. (1992) reported that octopus cells can display O_I or O_L response patterns. Oertel et al. (1990) described an octopus cell in an in vitro slice of the CN that

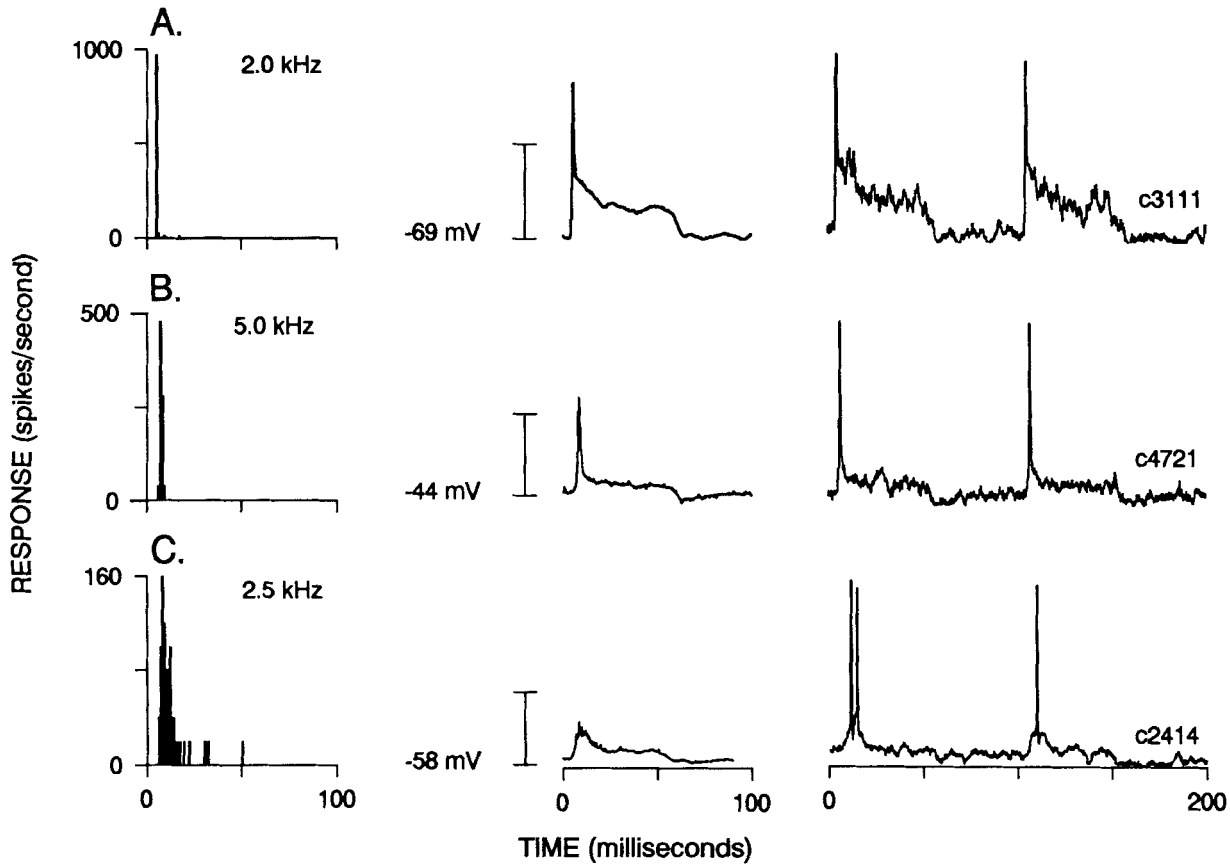


Fig. 9. Responses of three onset neurons. **Left column:** PSTHs: 1 msec bins, 50 ms tone bursts delivered every 100 ms. Number of stimulus repetitions of **A–C** are 75, 25, and 70, respectively. Stimulus level (re: threshold) for A and C is 40 dB, for B, 80 dB SPL. **Middle column:** Averaged responses from intracellular recordings to tone bursts. Averages are based on the same number of repetitions as in the left column. **Right column:** Intracellular recording to two tone bursts at the frequencies indicated in the left column. Resting potentials and vertical calibration (bars = 20 mV) are indicated in the middle column. A, C: Chinchilla. B: Gerbil. (Morphology of B shown in Ostapoff et al., 1994: Fig. 12B.)

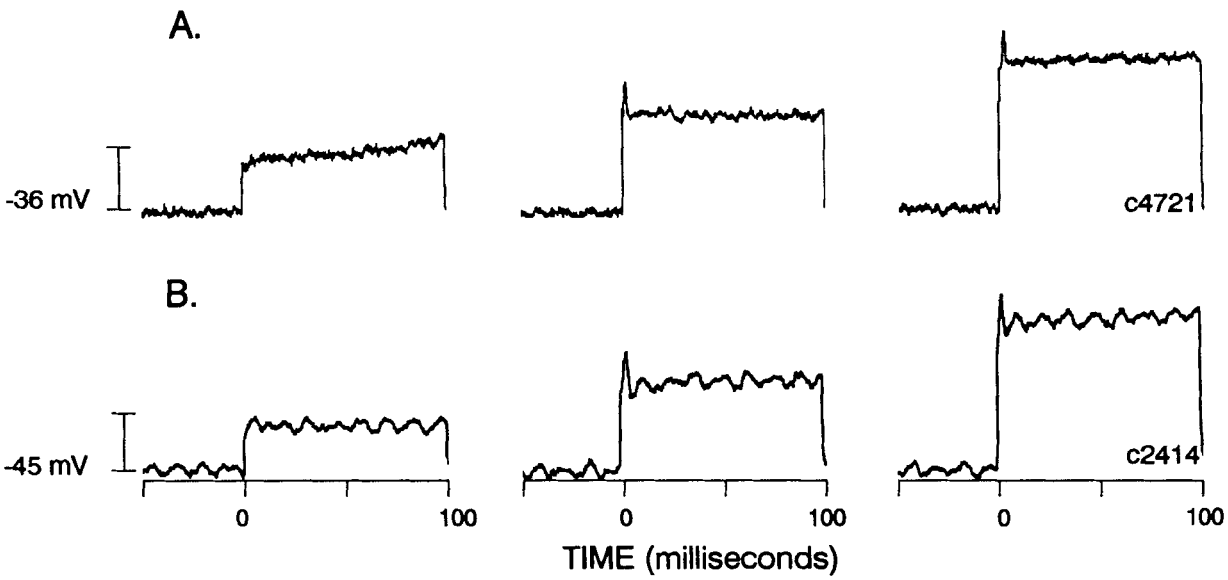


Fig. 10. Responses of two onset neurons (**A, B**) to increasing levels of depolarizing current (left to right: subthreshold, near-threshold, suprathreshold). Same format as in Figure 7. Vertical calibration bars = 20 mV.

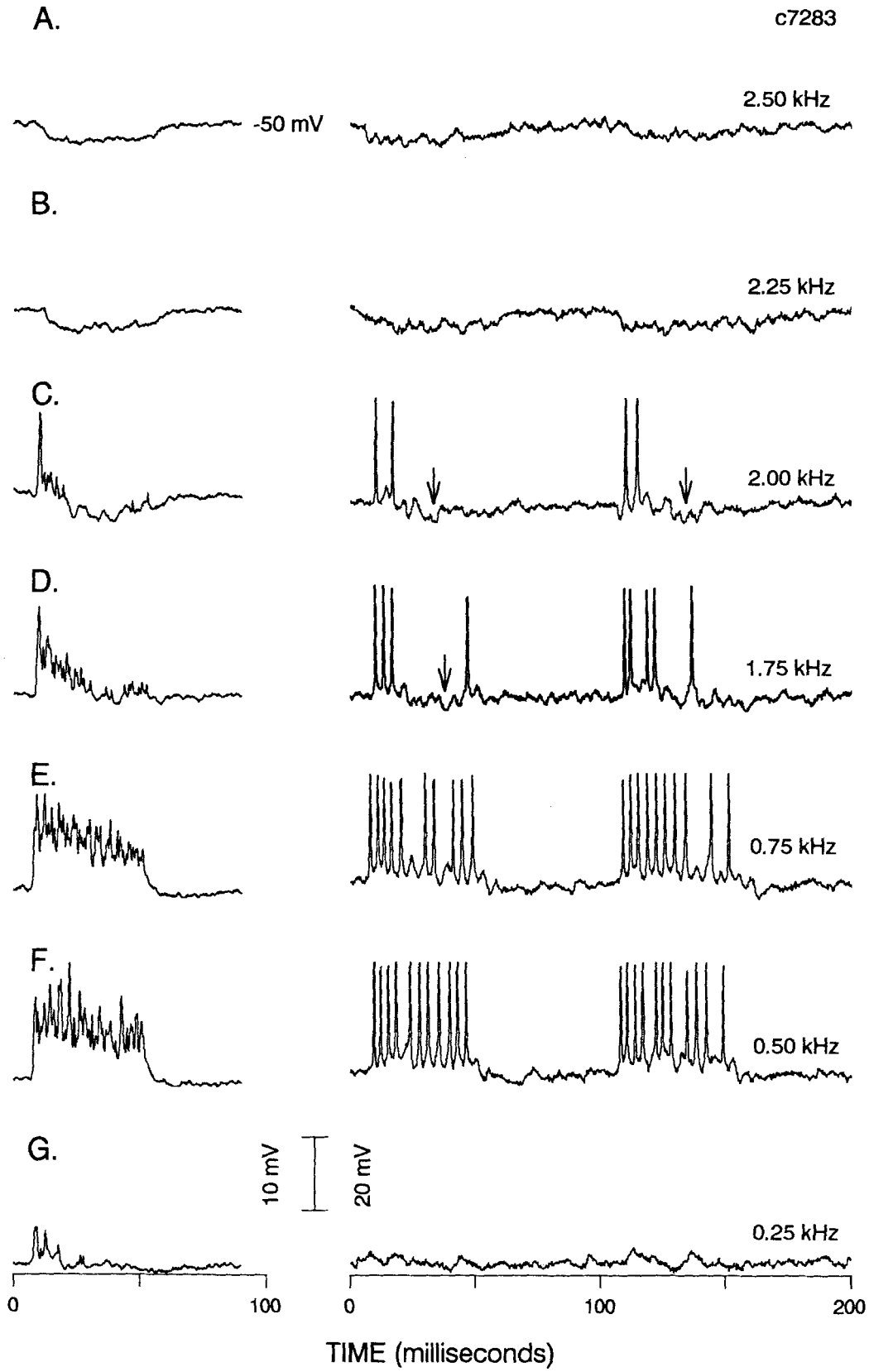
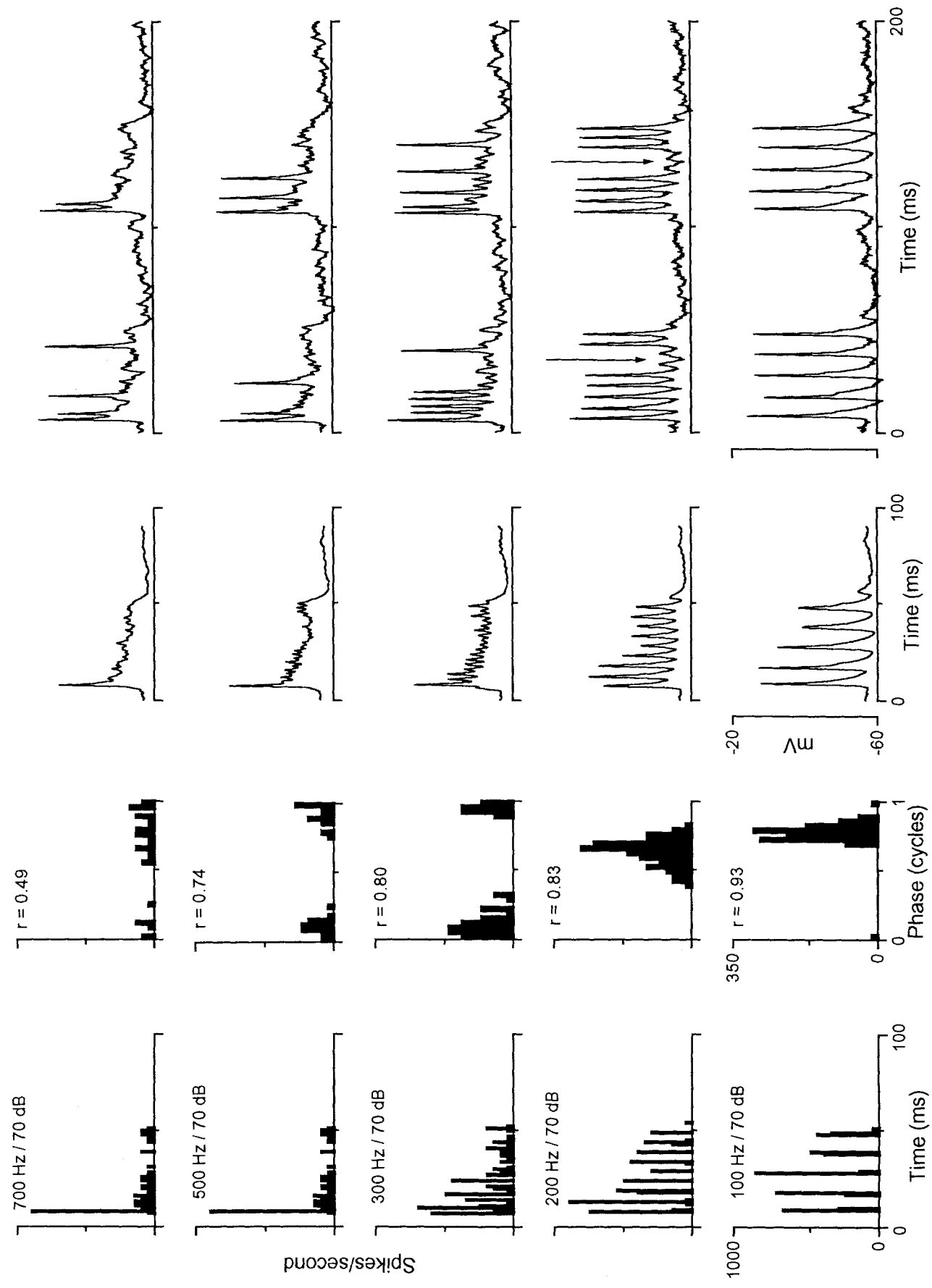


Fig. 11. **A–G:** Responses of a chop-T neuron (same as in Fig. 2D) at several frequencies (2.5–0.25 KHz). **Left column:** Averaged responses from intracellular recordings to tones: 50 ms tone bursts every 100 ms, repeated 20 times. Stimulus level (re: threshold) 20–30 dB. **Right column:** Intracellular recordings showing the responses of this neuron to two tone bursts at the indicated frequencies. Note hyperpolarization in A–C of both columns. Arrows indicate presumed inhibitory postsynaptic potentials (IPSPs). Resting potential is −50 mV. Vertical calibration bar = 10 mV for the averaged records, 20 mV for the individual ones.


700 Hz / 70 dB
500 Hz / 70 dB
300 Hz / 70 dB
200 Hz / 70 dB
100 Hz / 70 dB
Spikes/second
1000
0
Time (ms)
100
r = 0.49
r = 0.74
r = 0.80
r = 0.83
r = 0.93
350
0
Phase (cycles)
1
mV
-20
-60
Time (ms)
200

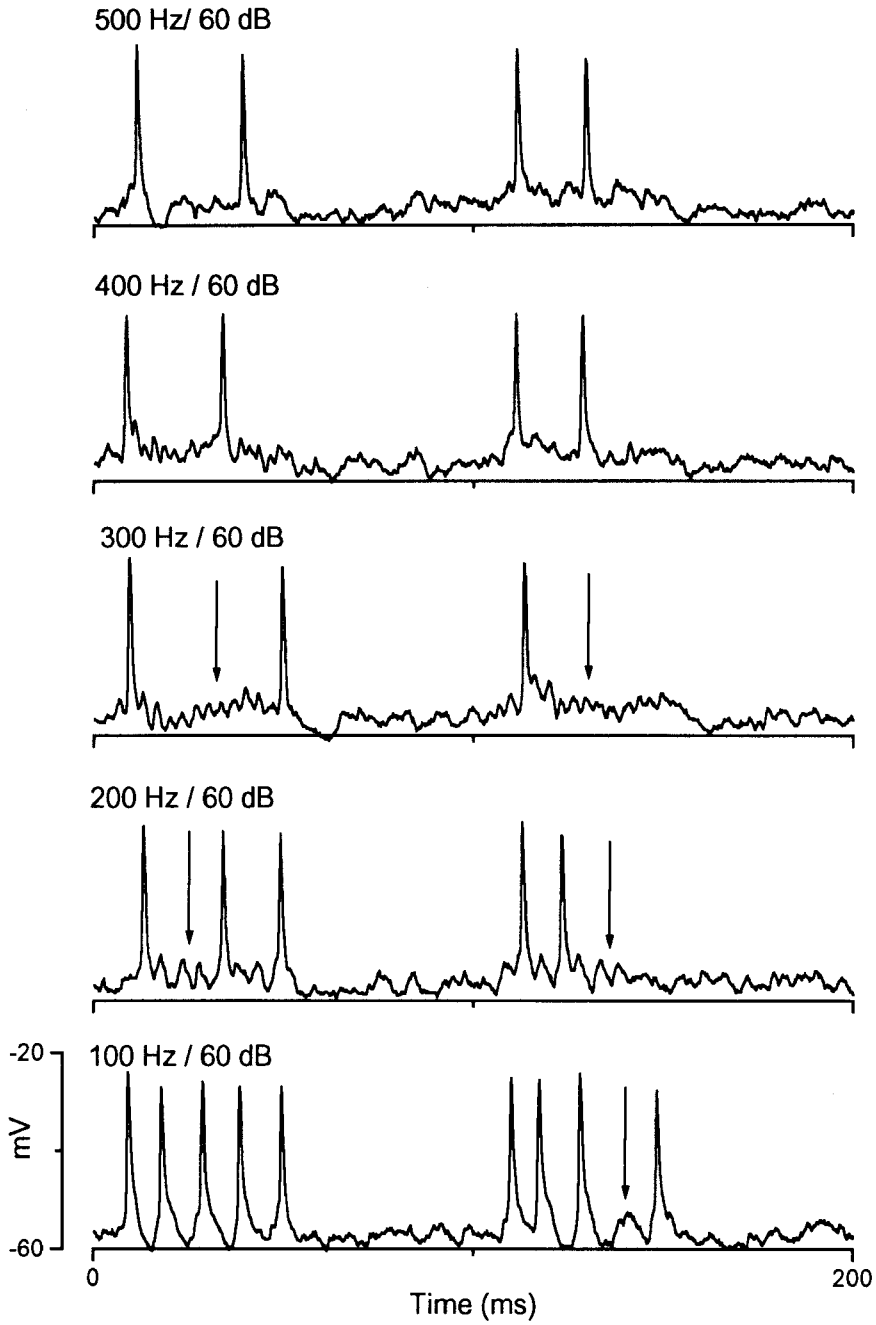


Fig. 13. Responses of the same phase-locked neuron in Figure 12 at several frequencies (100–500 Hz), only at a lower intensity (60 dB SPL). Shown are the intracellular responses to two sequential tone bursts at the indicated frequencies. Arrows indicate presumed EPSPs. Vertical calibration bar = 40 mV.

Fig. 12. Responses of a phase-locked neuron at several frequencies (100–700 Hz), all at 70 dB SPL. **First column:** PSTHs for 50 ms tone bursts, delivered every 100 ms, repeated 20 times at the frequencies indicated. **Second column:** Period histograms of responses for the period of the stimulating frequency were generated from the PSTHs in the first column. **Third column:** Averaged responses from intracellular recordings to tone bursts. **Fourth column:** Intracellular recordings showing the responses of this neuron to two tone bursts at the indicated frequencies. Arrows indicate presumed excitatory postsynaptic potentials (EPSPs). Vertical calibration bar = 40 mV for all intracellular traces, including the averaged records. (Morphology of this cell shown in Ostapoff et al., 1994: Fig. 16.)

had a sustained discharge to injected current. Recently, however, the same laboratory reported recordings from octopus cells that are consistent with our results (Golding et al., 1992). They found that octopus cells respond with a single action potential only at the start of a depolarizing current pulse.

Other response features

Inhibitory influences. The hyperpolarization observed in response to tones above best frequency for both a chop-T and a chop-S neuron suggests that inhibitory mechanisms

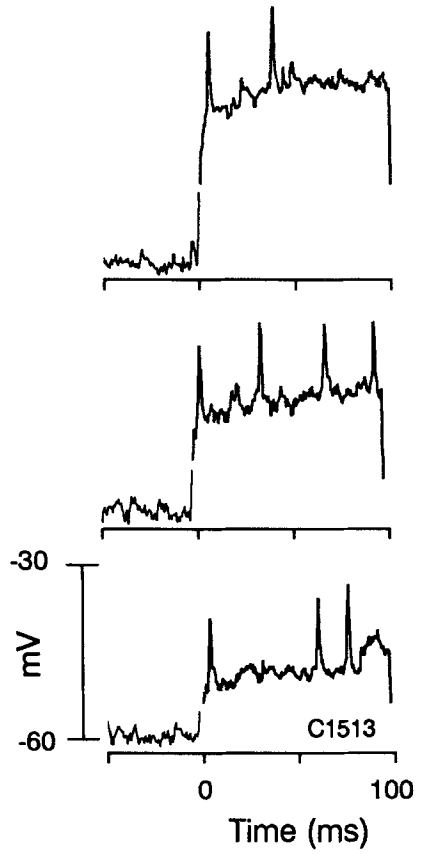


Fig. 14. Responses of the phase-locked neuron in Figures 12 and 13 to increasing levels of depolarizing current (bottom to top). Calibration bar = 30 mV.

operate in the ventral CN. Rhode and Smith (1986a) provided intracellular recordings of side-band inhibition in a chop-S neuron. Winter and Palmer (1990) reported similar results for choppers of both types from extracellular recordings. Thus, side-band inhibition alone cannot account for chop-T responses. Our data (Fig. 11) are consistent with the possibility that the inhibition observed above best frequency is also present in the responses at best frequency. Inhibitory inputs to choppers and onset choppers (O_c; Rhode and Smith, 1986a) may shape their responses to stimuli near their best frequency (Palombi and Caspary, 1992). Inhibition at best frequency has been postulated for chop-T neurons (Blackburn and Sachs, 1992) and has been used in models of chopper cells to produce chop-T responses (Arle and Kim, 1991; Banks and Sachs, 1991). In the posteroventral CN, Smith and Rhode (1989) demonstrated that a neuron with an O_C response, which they suggest may be analogous to a chop-T response, had more putative inhibitory somatic inputs (i.e., terminals containing nonspherical vesicles) than chop-S neurons. The type and magnitude of axosomatic inputs may underlie the difference between chop-S and chop-T neurons, but there are other possibilities.

Side-band inhibition might be linked to an endogenous circuit based on its short latency (Fig. 11). There is strong electrophysiological evidence that certain neurons in the dorsal CN can produce a short latency inhibition in ventral CN neurons (Wickesberg and Oertel, 1990). Possible sources of inhibitory circuits would include projections, using glycine or gamma-aminobutyric acid from the dorsal CN (Wickesberg and Oertel, 1990; Saint Marie et al., 1991; Osen et al., 1990) and the periolivary nuclei (Potashner et al., 1985; Statz-Benson and Potashner, 1988; Benson and Potashner, 1990; Ostapoff et al., 1992). Multisynaptic circuits might involve interneurons using a variety of neurotransmitters, e.g., acetylcholine, including cells located in the periolivary nuclei, the dorsal CN, and the ventral CN (Godfrey et al., 1990). Finally, axon collaterals of the olivocochlear bundle could contribute to mono- or multisynaptic inhibitory circuits (Rasmussen, 1960, 1967).

Phase locking. One neuron was distinguished by highly effective and precise phase locking at low frequencies, while it had an onset pattern at higher frequencies (Figs. 12–14). Although its irregular responses to current resembled those of the primary-like neurons, it differed from these in its ability to sustain a depolarization and in its PSTH. The response of this cell must not have been inherited from its input, since cycle-by-cycle phase locking is not known to occur in the cochlear nerve (Rose et al., 1967). Such phase locking, associated with onset discharge patterns, has been reported for neurons in the posteroventral CN (Godfrey et al., 1975; Rhode and Smith, 1986a).

The highest frequency at which the cell of Figures 12–14 could phase lock is likely to be determined by the time constant of its membrane, since the way the graded potential changed with frequency is what might be expected to happen if it had been passed through a low-pass filter. At the lowest frequencies, where the period of the tone is long, the graded potential appears to consist of EPSPs, each following one cycle of the tone. At higher frequencies (about 300 Hz), individual EPSPs merged but were still seen in the phase-locked oscillations superimposed on a sustained depolarization. At still higher frequencies, the amplitude of the oscillation declined. Some of the membrane properties of this neuron may resemble those of the onset neurons that we studied, in that both appear to be capable of sustaining a depolarization. However, the sustained depolarization seen at higher frequencies, unlike that of choppers, does not evoke a continuing train of action potentials. Instead, action potentials occur mostly during the initial portion of the stimulus. This may result from an inactivation process, which we have already invoked for the onset neurons. The inactivation process, associated with a sustained depolarization and the time constant of the membrane, may reflect a general mechanism in neurons with onset discharge patterns at higher frequencies and cycle-by-cycle phase locking at lower frequencies.

SUMMARY

While neurons in the CN receive relatively homogenous inputs from the cochlear nerve, their morphology is diverse and their response patterns are heterogeneous. Comparing intracellular responses to tones and depolarizing current in the same cells provides evidence that the membrane properties of different types of neurons play a role in determining the temporal pattern of their outputs.

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LITERATURE CITED

Adams, J.C. (1976) Single unit studies on the dorsal and intermediate acoustic striae. J. Comp. Neurol. *170:*97–106.

Arle, J.E., and D.O. Kim (1991) Neural modeling of intrinsic and spike-discharge properties of cochlear nucleus neurons. Biol. Cybern. *64:*273–283.

Banks, M.I., and M.B. Sachs (1991) Regularity analysis in a compartment model of chopper units in the anteroventral cochlear nucleus. J. Neurophysiol. *65:*606–629.

Banks, M.I., and P.H. Smith (1992) Intracellular recordings from neurobiotin-labeled cells in brain slices of rat medial nucleus of the trapezoid body. J. Neurosci. *12:*2819–2837.

Benson, C.G., and S.J. Potashner (1990) Retrograde transport of [^{3}H] glycine from the cochlear nucleus to the superior olive in the guinea pig. J. Comp. Neurol. *296:*415–426.

Blackburn, C.C., and M.B. Sachs (1989) Classification of unit types in the anteroventral cochlear nucleus: PST histograms and regularity analysis. J. Neurophysiol. *62:*1303–1329.

Blackburn, C.C., and M.B. Sachs (1992) Effects of off-BF tones on the responses of chopper units in the ventral cochlear nucleus. J. Neurophysiol. *68:*124–143.

Bourk, T.R. (1976) Electrical responses of neural units in the anteroventral cochlear nucleus of the cat. Ph.D. Thesis. MIT, Cambridge, MA.

Bourk, T.R., J.P. Mielcarz, and B.E. Norris (1981) Tonotopic organization of the anteroventral cochlear nucleus of the cat. Hearing Res. *4:*215–241.

Brawer, J.R., and D.K. Morest (1975) Relations between auditory nerve endings and cell types in the cat anteroventral cochlear nucleus seen with the Golgi method and Nomarski optics. J. Comp. Neurol. *160:*491–506.

Brawer, J.R., D.K. Morest, and E.C. Kane (1974) The neuronal architecture of the cochlear nucleus of the cat. J. Comp. Neurol. *155:*251–300.

Cant, N.B., and D.K. Morest (1979) The bushy cells in the anteroventral cochlear nucleus of the cat. A study with the electron microscope. Neuroscience *4:*1925–1945.

Feng, J.J. (1989) The coding of sound in the cochlear nucleus: Mechanisms underlying the generating of response patterns by different morphological cell types. Ph.D. Thesis. The University of Connecticut, Farmington, CT.

Frisina, R.D., S.C. Chamberlain, M.L. Brachman, and R.L. Smith (1982) Anatomy and physiology of the gerbil cochlea nucleus: An improved surgical approach for microelectrode studies. Hearing Res. *6:*259–276.

Godfrey, D.A., N.Y.-S. Kiang, and B.E. Norris (1975) Single unit activity in the posteroventral cochlear nucleus of the cat. J. Comp. Neurol. *162:*247–268.

Godfrey, D.A., K.L. Beranek, L. Carlson, J.A. Parli, J.D. Dunn, and D.R. Ross (1990) Contributions of centrifugal innervation to choline acetyltransferase activity in the cat cochlear nucleus. Hearing Res. *49:*259–280.

Goldberg, J.M., and P.B. Brown (1969) Responses of binaural neurons of dog superior olivary complex to dichotic tonal stimuli: Some physiological mechanisms of sound localization. J. Neurophysiol. *32:*613–634.

Golding, N.I., D. Robertson, R.E. Wickesberg, and D. Oertel (1992) Intracellular recordings from octopus cells in cochlear nuclei of mice in slices. Soc. Neurosci. Abstr. *18:*151.

Granit, R., and C.G. Phillips (1956) Excitatory and inhibitory processes acting upon individual Purkinje cells of the cerebellum in cats. J. Physiol. (Lond.) *133:*520–547.

Hirsch, J.A., and D. Oertel (1988a) Intrinsic properties of neurones in the dorsal cochlear nucleus of mice, *in vitro*. J. Physiol. (Lond.) *396:*535–548.

Hirsch, J.A., and D. Oertel (1988b) Synaptic connections in the dorsal cochlear nucleus of mice, *in vitro*. J. Physiol. (Lond.) *396:*549–562.

Hodgkin, A.L., and A.F. Huxley (1952) The dual effect of membrane potential on sodium conductance in the giant axon of Loligo. J. Physiol. (Lond.) *116:*497–506.

Joris, P.X., P.H. Smith, and T.C.T. Yin (1992) The responses and projections of dorsal and intermediate stria axons, labeled with HRP or neurobiotin. Assoc. Res. Otolaryngol. Abstr. *15:*58.

Kane, E.C. (1973) Octopus cells in the cochlear nucleus of the cat: Heterotypic synapses upon homeotypic neurons. Int. J. Neurosci. *5:*251–279.

Kiang, N.Y.-S., T. Watanabe, E.C. Thomas, and L.F. Clark (1965) Discharge Patterns of Single Fibers in the Cat's Auditory Nerve. Cambridge, MA: M.I.T. Press, Res. Monograph #35.

Kiang, N.Y.-S., D.K. Morest, D.A. Godfrey, J.J. Guinan, Jr., and E.C. Kane (1973) Stimulus coding at caudal levels of the cats auditory nervous system: I. Response characteristics of single units. In A.R. Møller (ed): Basic Mechanisms in Hearing. New York: Academic Press, pp. 455–477.

Manis, P.B. (1990) Membrane properties and discharge characteristics of guinea pig dorsal cochlear nucleus neurons studied *in vitro*. J. Neurosci. *10:*2338–2351.

Manis, P.B., and S.O. Marx (1991) Outward currents in isolated ventral cochlear nucleus neurons. J. Neurosci. *11:*2865–2880.

Morest, D.K., N.Y.-S. Kiang, E.C. Kane, J.J. Guinan, Jr., and D.A. Godfrey (1973) Stimulus coding at caudal levels of the cats auditory nervous system: II. Patterns of synaptic organization. In A.R. Møller (ed): Basic Mechanisms in Hearing. New York: Academic Press, p. 479.

Oertel, D. (1983) Synaptic responses and electrical properties of cells in brain slices of the mouse anteroventral cochlear nucleus. J. Neurosci. *3:*2043–2053.

Oertel, D., and S.H. Wu (1989) Morphology and physiology of cells in slice preparations of the dorsal cochlear nucleus of mice. J. Comp. Neurol. *283:*228–247.

Oertel, D., S.H. Wu, M.W. Garb, and C. Dizack (1990) Morphology and physiology of cells in slice preparations of the posteroventral cochlear nucleus of mice. J. Comp. Neurol. *295:*136–154.

Ogden, T.E., M.C. Citron, and R. Pieranto (1978) Jet stream microbeveler—inexpensive way to bevel ultrafine glass micropipettes. Science *201:*469–470.

Osen, K.K., O.P. Ottersen, and J. Storm-Mathisen (1990) Co-localization of glycine-like and GABA-like immunoreactivities: A semiquantitative study of individual neurons in the dorsal cochlear nucleus of cat. In O.P. Ottersen and J. Storm-Mathisen (eds): Glycine Neurotransmission. New York: John Wiley & Sons, pp. 417–451.

Ostapoff, E.-M., C.G. Benson, and R.L. Saint Marie (1992) Superior olivary projections to the dorsal and anteroventral cochlear nucleus in guinea pig differ in the patterns of GABA and glycine immunoreactivities. Assoc. Res. Otolaryngol. Abstr. *15:*57.

Ostapoff, E.-M., J.J. Feng, and D.K. Morest (1994) A physiological and structural study of neuron types in the cochlear nucleus. II. Neuron types and their structural correlations with response properties. J. Comp. Neurol. *346:*19–42.

Palombi, P.S., and D.M. Caspary (1992) The GABA-A receptor antagonist bicuculline alters the response properties of posteroventral cochlear nucleus neurons. J. Neurophysiol. *67:*738–746.

Parham, K., and D.O. Kim (1992) Analysis of temporal discharge characteristics of dorsal cochlear nucleus neurons of unanesthetized decerebrate cats. J. Neurophysiol. *67:*1247–1263.

Pfeiffer, R.R. (1966) Classification of response patterns of spike discharge for units in the cochlear nucleus: Tone-burst stimulation. Exp. Brain. Res. *1:*220–235.

Potashner, S.J., N. Lindberg, and D.K. Morest (1985) Uptake and release of γ-aminobutyric acid in the guinea pig cochlear nucleus after axotomy of cochlear and centrifugal fibers. J. Neurochem. *45:*1558–1566.

Rasmussen, G.L. (1960) Efferent fibers of the cochlear nerve and cochlear nucleus. In G.L. Rasmussen and W.F. Windle (eds): Neural Mechanisms of Auditory and Vestibular Systems. Springfield: Thomas, pp. 105–115.

Rasmussen, G.L. (1967) Anatomic relations of the ascending and descending auditory systems. In W.S. Fields and B.R. Alford (eds): Neurological Aspects of Auditory and Vestibular Disorders. Springfield: Thomas, pp. 5–19.

Rhode, W.S., and P.H. Smith (1986a) Encoding timing and intensity in the ventral cochlear nucleus of the cat. J. Neurophysiol. *56:*261–286.

Rhode, W.S., and P.H. Smith (1986b) Physiological studies on neurons in the dorsal cochlear nucleus of the cat. J. Neurophysiol. *56:*287–307.

Rhode, W.S., P.H. Smith, and D. Oertel (1983a) Physiological response properties of cells labelled intracellularly with horseradish peroxidase in cat dorsal cochlear nucleus. J. Comp. Neurol. *213:*426–447.

Rhode, W.S., D. Oertel, and P.H. Smith (1983b) Physiological response properties of cells labelled intracellularly with horseradish peroxidase in cat ventral cochlear nucleus. J. Comp. Neurol. *213:*448–463.

Ritz, L.A., and W.E. Brownell (1982) Single unit analysis of the posteroventral cochlear nucleus of the decerebrate cat. Neuroscience *7:*1995–2010.

Romand, R. (1978) Survey of intracellular recording in the cochlear nucleus of the cat. Brain Res. *148:*43–65.

Rose, J.E., J.F. Brugge, D.J. Anderson, and J.E. Hind (1967) Phase-locked response to low-frequency tones in single auditory nerve fibers of the squirrel monkey. J. Neurophysiol. *30:*769–793.

Rouiller, E.M., and D.K. Ryugo (1984) Intracellular marking of physiologically characterized cells in the ventral cochlear nucleus of the cat. J. Comp. Neurol. *225:*167–186.

Saint Marie, R.L., C.G. Benson, E.-M. Ostapoff, and D.K. Morest (1991) Glycine immunoreactive projections from the dorsal to the anteroventral cochlear nucleus. Hearing Res. *51:*11–28.

Smith, P.H., and W.S. Rhode (1985) Electron microscopic features of physiologically characterized, HRP-labelled fusiform cells in the cat dorsal cochlear nucleus. J. Comp. Neurol. *237:*127–143.

Smith, P.H., and W.S. Rhode (1987) Characterization of HRP-labelled globular bushy cells in the cat anteroventral cochlear nucleus. J. Comp. Neurol. *266:*360–375.

Smith, P.H., and W.S. Rhode (1989) Structural and functional properties distinguish two types of multipolar cells in the ventral cochlear nucleus. J. Comp. Neurol. *282:*595–616.

Staatz-Benson, C., and S.J. Potashner (1988) Uptake and release of glycine in the guinea pig cochlear nucleus after axotomy of afferent or centrifugal fibers. J. Neurochem. *51:*370–379.

Tolbert, L.P., and D.K. Morest (1982) The neuronal architecture of the anteroventral cochlear nucleus of the cat in the region of the cochlear nerve root: Golgi and Nissl methods. Neuroscience *7:*3013–3030.

Tolbert, L.P., D.K. Morest, and D.A. Yurgelun-Todd (1982) The neuronal architecture of the anteroventral cochlear nucleus of the cat in the region of the cochlear nerve root: Horseradish peroxidase labeling of identified cell types. Neuroscience *7:*3031–3052.

Wickesberg, R.E., and D. Oertel (1990) Delayed, frequency specific inhibition in the cochlear nucleus of mice: A mechanism for monaural echo suppression. J. Neurosci. *10:*1762–68.

Winter, I.M., and A.R. Palmer (1990) Responses of single units in the anteroventral cochlear nucleus of the guinea pig. Hearing Res. *44:*161–178.

Wu, S.H., and D. Oertel (1984) Intracellular injection with horseradish peroxidase of physiologically characterized stellate and bushy cells in slices of mouse anteroventral cochlear nucleus. J. Neurosci. *4:*1577–1588.

Young, E.D., J.-M. Robert, and W.P. Shofner (1988) Regularity and latency of units in ventral cochlear nucleus: Implications for unit classification and generation of response properties. J. Neurophysiol. *60:*1–29.