Intracellular Responses of Onset Chopper Neurons in the Ventral Cochlear Nucleus to Tones: Evidence for Dual-Component Processing

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Paolini, Antonio G. and Graeme M. Clark. Intracellular responses of onset chopper neurons in the ventral cochlear nucleus to tones: evidence for dual-component processing. J. Neurophysiol. 81: 2347–2359, 1999. The ventral cochlear nucleus (VCN) contains a heterogeneous collection of cell types reflecting the multiple processing tasks undertaken by this nucleus. This in vivo study in the rat used intracellular recordings and dye filling to examine membrane potential changes and firing characteristics of onset chopper (Oc) neurons to acoustic stimulation (50 ms pure tones, 5 ms rise time). Stable impalements were made from 15 Oc neurons, 7 identified as multipolar cells. Neurons responded to characteristic frequency (CF) tones with sustained depolarization below spike threshold. With increasing stimulus intensity, the depolarization during the initial 10 ms of the response became peaked, and with further increases in intensity the peak became narrower. Onset spikes were generated during this initial depolarization. Tones presented below CF resulted in a broadening of this initial depolarizing component with high stimulus intensities required to initiate onset spikes. This initial component was followed by a sustained depolarizing component lasting until stimulus cessation. The amplitude of the sustained depolarizing component was greatest when frequencies were presented at high intensities below CF resulting in increased action potential firing during this period when compared with comparable high intensities at CF. During the presentation of tones at or above the high-frequency edge of a cell’s response area, hyperpolarization was evident during the sustained component. The presence of hyperpolarization and the differences seen in the level of sustained depolarization during CF and off CF tones suggests that changes in membrane responsiveness between the initial and sustained components may be attributed to polysynaptic inhibitory mechanisms. The dual-component processing resulting from convergent auditory nerve excitation and polysynaptic inhibition enables Oc neurons to respond in a unique fashion to intensity and frequency features contained within an acoustic stimulus.

INTRODUCTION

The cochlear nucleus is the first relay center of the central auditory pathway. It can be subdivided into ventral and dorsal divisions containing cells that receive direct input from the auditory nerve (Lorente de Nó 1933, 1981). The cells contained within the cochlear nucleus can be divided into classes based on their unitary response to sound (Kiang et al. 1965; Pickles 1988; Rhode and Smith 1986; Winter and Palmer 1990). Past studies also correlated neural response with morphology with intracellular recording and dye filling (Feng et al. 1994; Ostapoff et al. 1994; Rhode et al. 1983; Roullier and Ryugo 1984; Smith and Rhode 1987, 1989). Although various classes of onset neurons were described in the ventral cochlear nucleus (VCN), this investigation focuses on the onset chopper type (Oc) described by Rhode and Smith (1986).

Oc units respond to acoustic tones with an initial series of spikes, with regular intervals, unrelated to stimulus frequency, which are followed by varying degrees of sustained activity for the duration of the tone (Rhode and Smith 1986). Oc neurons are also unique in that they respond over a wide frequency range and have a large dynamic range. It was suggested that these neurons play an important role in the processing of complex sounds such as speech in addition to coding intensity (Rhode and Smith 1986; Romand and Avan 1997). Sound localization was also proposed as a function of Oc neurons (Romand and Avan 1997) because of a small variability in first spike latency seen across the Oc unit population (Rhode and Smith 1986). This small variability in first spike latency may be related to the extent to which these neurons receive auditory nerve input. Jiang et al. (1996) demonstrated that the two tone facilitation effects of Oc units in the VCN observed by Winter and Palmer (1995) occur over a wide range of frequencies, suggesting auditory nerve input from a broad region of the basilar membrane.

The Oc–responding units were morphologically identified as multipolar (stellate) cells. Two types of multipolar or stellate cells were described in the VCN, distinguished by the extent of somatic innervation (Cant 1981; Smith and Rhode 1989). Multipolar cells described as having an Oc response receive the bulk of their innervation onto the cell soma (Smith and Rhode 1989). They have axons containing pleomorphic vesicles and terminate within the VCN, suggesting that they are inhibitory. There is also evidence to suggest that they may terminate on multipolar cells with sparse somatic input (Smith and Rhode 1989). These two multipolar neuron types were termed D- and T-stellate cells, respectively, by Oertel et al. (1990). Recent evidence supports inhibitory contact from D- to T-stellate cells (Ferragamo et al. 1998). Inhibition was thought of as being responsible for shaping cochlear nucleus neuron responses to sound (Britt and Starr 1976; Starr and Britt 1970). The possible inhibitory nature of D-stellate neurons and their wide frequency response area suggest that they may play an important role in regulating sound processing within the VCN.

In this investigation, we aim to examine the neural mecha-
nisms that may underlie the O\textsubscript{C} response with in vivo intracellular recording techniques. Because of the VCN’s position within the brain stem, it often proved difficult to obtain good intracellular impalements in vivo. We examined the intracellular neural response of these neurons to tones presented both on and off characteristic frequency (CF) and over a wide intensity range.

METHODS

Preparation

All experiments were performed on 11 Long-Evans rats weighing 240 to 300 g. They were anesthetized with urethan in water (1.3 g/kg ip) and were breathing spontaneously. Supplemental doses were administered if at any time during the experiment a corneal or paw reflex was observed. All efforts were made to prevent any animal suffering, and these procedures were in accordance with the Royal Victorian Eye and Ear Hospital Animal Research Ethics Committee guidelines (Project 95037).

After a craniotomy, the cerebellum was aspirated on one side to expose the cochlear nucleus, enabling intracellular recording electrodes to be inserted into the VCN under visual control (Paolini and Clark 1988). Core body temperature was maintained at ~37°C with a DC homoeothermic blanket.

Recording

Microelectrodes, made from thin-walled (1.0-mm OD) quartz glass (Sutter Instrument, CA), were filled with 1 M potassium acetate (70–80 MΩ) or in some cases 4% Neurobiotin in 1 M potassium acetate and advanced through the VCN. When stable cell impalements were obtained, acoustic stimuli were delivered to the animal. Intracellular stable impalements were signaled by a prolonged (>3 min), stable drop (>30 mV) in the DC level and the presence of synaptic or large action potentials (>20 mV) with monophasic rise and fall times. Recordings were possible for ≤140 min. A MacLab 4S data acquisition system (AD Instruments) was used to store electrophysiological traces typically at a bandwidth of 20 or 40 kHz. Acoustic stimuli were synthesized digitally and generated by a Beyer DT48 transducer, which was positioned at the end of a hollow ear bar and controlled with a PDP-11/34 computer. The acoustic system was calibrated with a Bruel & Kjaer (B&K) measuring amplifier (type 2606) and a second B&K ½-in. condenser microphone coupled to a small probe tube positioned within the ear bar tube ~3 mm from the tympanic membrane to enable acoustic input to be measured in dB SPL.

Once impaled, the neuron’s CF and acoustic spike input–output functions to frequencies at or away from CF were determined. Neuron response area was obtained from these input–output functions. The CF was calculated from an acoustic threshold tuning curve that was constructed on-line as described in detail by Liberman (1978). Acoustic input–output functions were constructed initially at the CF. The acoustic stimulus was delivered in 5-dB steps in a sequential manner from subthreshold to saturation intensity. With each increase in intensity, ≤50 repetitions of the stimulus were presented. The acoustic stimulus typically consisted of a 50-ms pure tone burst with a 5-ms rise and fall time and a 5-Hz repetition frequency. Peristimulus time (PST) and interspike interval (ISI) histograms at 0.1-ms binwidths. Bin size = 0.1 ms.

Histology

The cochlear nucleus was examined histologically for Neurobiotin-filled cells. After urethan anesthetic overdose the rats were perfused transcardially with 10% formalin containing 30% sucrose. The head was removed and preserved in the sucrose–formalin solution. After 2 days the brain stem was removed, and the cochlear nucleus was sectioned at 120 μm parasagittally on a freezing microtome. Brain sections were processed with avidin–horseradish peroxidase (HRP) intensified with 3,3′-diaminobenzidine (DAB) (Adams 1981).

Data analysis

Cells were traced from 120-μm sections with camera lucida as described by Paolini and Clark (1998). Cells that could be identified

FIG. 1. Typical peristimulus time (PST) histograms and interspike interval (ISI) histograms shown by onset chopper (O\textsubscript{C}) neurons (A–D). Histograms outline spike response features to a 50-ms tone presented at characteristic frequency (CF) over 50 repetitions. The initial peak was usually followed by 1–4 peaks followed by varying degrees of sustained activity. The rate-level curves for these neurons are shown in E. CF and intensity indicated on histograms.

Data analysis

Cells were traced from 120-μm sections with camera lucida as described by Paolini and Clark (1998). Cells that could be identified
and traced in continuity were reconstructed from serial sections with a drawing tube at a total magnification of ×625 with an oil-immersion lens (planachromat, NA 1.0).

RESULTS

Intracellular recordings

Fifteen O\textsubscript{C} neurons were identified based on their intracellular response properties to tones. Onset neurons had a mean (±SE) resting membrane potential of \(-56.1 \pm 3.2\) mV with a mean action potential amplitude (with corresponding ranges) of \(39.2 \pm 3.5\) mV (22–60 mV). The mean CF was \(11.6 \pm 1.2\) kHz (3.6–20 kHz) with a mean threshold intensity of \(50.8 \pm 4.8\) dB SPL (25–69 dB SPL).

\(O\textsubscript{C}\) response type

Typical PST histograms, ISI histograms, and rate-level curves are shown in Fig. 1. As described by Rhode and Smith (1986) and Smith and Rhode (1989), \(O\textsubscript{C}\) neurons are characterized by a well-timed first peak in the PST histogram. For the duration of the tone, this first peak was rapidly followed by one to four less-well-timed successive peaks followed by sustained activity of varying degrees (Fig. 1, A–D). The spike time CV verified \(O\textsubscript{C}\) behavior. During the initial period of the stimulus the neurons had CVs <0.4 (regular firing), which increased to >0.4 during the middle to late stages of the stimulus (Fig. 2).

The width of tuning was examined in four neurons (235–003, 244–009, 249–012, and 257–002) with similar CFs of ∼10 kHz by measuring \(Q\textsubscript{10}\) (CF/bandwidth). The average estimate of \(Q\textsubscript{10}\) for these neurons was 3.1 ± 0.2 (2.5–3.6).

Intracellular response characteristics at CF

Neurons defined as \(O\textsubscript{C}\) also showed similar intracellular responses to tones presented at CF. At just above response threshold, \(O\textsubscript{C}\) neurons responded to tones at CF with a sustained depolarization throughout the stimulus period. As the intensity of the acoustic stimulus was increased the amplitude of the depolarization increased, particularly during the initial 10 ms of stimulus presentation. Figure 3 shows responses of an identified VCN multipolar cell to tones presented at CF. This neuron had a CF of 10.8 kHz responding in an \(O\textsubscript{C}\) manner at this frequency (Fig. 1A).

FIG. 2. CV calculated for 6 cells at 10-ms intervals (over 50 repetitions) during CF tone presentation. Numbers corresponding to each plot indicate stimulus intensity (dB SPL). Neuron number and CF shown at top left-hand corner.

FIG. 3. Intracellular response to 50-ms tones presented at CF (10.8 kHz). A: representative examples of neuron response to tones from depolarization threshold to saturation intensity. B: average intracellular response of the neuron over 25 repetitions of the CF tone presented at intensities shown in A. An initial and sustained component was evident in the response to acoustic stimuli indicated by circle and square, respectively. Action potential generation predominantly occurred during the initial phase of the response, which is truncated in B because of expanded vertical axis. Action potentials were followed by a short-duration hyperpolarizing afterpotential (arrow). Dotted line indicates the time at which the sustained depolarization began to return to resting levels. The duration and envelope of the 50-ms tone are shown in A and B, bottom.
At spike threshold, O\(_C\) neurons responded with predominantly one action potential occurring during the initial component of the depolarizing response, which was followed by a short-duration hyperpolarizing afterpotential (Fig. 3). As intensities increased from spike threshold an additional action potential was commonly initiated during this initial phase (Fig. 3). After the initial increase in depolarization, the membrane potential returned to a sustained depolarized level for the remainder of the stimulus presentation (Fig. 3). During this sustained component of the depolarizing response, spike generation was less frequent, particularly in neurons displaying low levels of spontaneous activity. Depolarization during this sustained component at CF was mostly below spike threshold.

Frequency and intensity effects at and off CF

The effect of changing intensity and frequency on the initial component is depicted in Fig. 4, A and B. The amplitude of initial depolarization increased with increasing stimulus intensity, particularly evident at CF and frequencies just above or below CF (Fig. 4, A and B; 10.8, 11.8, and 3.8 kHz). During the initial component, a higher tone intensity was required below CF to produce an equivalent depolarization amplitude to that seen at CF. The amplitude rise associated with this initial component had a 20- to 25-dB range before consistent action potential generation.

The effect of changing intensity and frequency on the sustained depolarization is shown in Fig. 4C. The amplitude change in depolarization from threshold to 20 to 25 dB above threshold was greatest below CF (Fig. 4C; 3.8 kHz). When intensity was kept constant, the amplitude of the sustained activity was greatest at frequencies below CF, particularly at the low-frequency edge of the neuron’s response area, and decreased with increasing frequency (Fig. 5). At frequencies well above CF (Fig. 4C; 14.8 kHz) an increase in intensity had little effect on the amplitude of the sustained portion of the response. Frequency presentations at or above CF also evoked a short transition time from the initial depolarization (measured from its peak) to sustained levels when compared with the longer transition times observed with below-CF presentations (Fig. 4C).

Contour maps in Fig. 6 display the dependence on intensity and frequency of the amplitude of depolarization during both the initial and sustained portion of the tone-evoked response in two O\(_C\) neurons with similar CFs. Both neurons show similar relationships. The level of depolarization during the initial component was maximum at CF for these two units (Fig. 6A). However, for the sustained component, the maximum depolarization tended to occur below CF at high intensities (Fig. 6B). At frequencies below CF these neurons were still able to respond with increases in depolarization amplitudes with increasing stimulus intensity (Fig. 6, A and B). This relationship was not observed when tones were presented with frequencies...
above CF, where the maximum amplitude of depolarization depended predominantly on frequency, changing little with increasing intensity (Fig. 6, A and B).

**Frequency and intensity effects on spike activation**

In O$_C$ neurons, because of the nature of the depolarization, action potentials evoked by tones occurred predominantly on the initial component of the depolarization. Depolarization rise times and first spike latency both decreased with increasing tone intensity for a given frequency presentation (Figs. 4, A and B, and 7). Although first spike latency may in part be dependent on the rise time of this excitation, the presence of a 5-ms onset ramp associated with tones may also contribute to this observed decrease in first spike latency with increases in stimulus intensity. The variability in first spike latency also decreased with increasing stimulus intensity for frequencies at or away from CF (Fig. 7, indicated by the error bars). At frequencies below CF the intensity required for initial spike activation was greater than that required for spike generation at CF (Fig. 7). Presentation of tones below CF also resulted in longer spike latencies at comparable intensities at or away from CF (Fig. 7, indicated by the error bars). At frequencies below CF the intensity required for initial spike activation was greater than that required for spike generation at CF (Fig. 7). Presentation of tones below CF also resulted in longer spike latencies at comparable intensities at or away from CF (Fig. 7, indicated by the error bars). At frequencies below CF the intensity required for initial spike activation was greater than that required for spike generation at CF (Fig. 7).

The average number of spike discharges per repetition increased with increasing intensity at and below CF. At frequencies above CF little change in firing rate was seen with changes in tone intensity (Fig. 8). Although the lowest intensity required for spike activation occurred at CF, the maximum discharge rate often occurred at high intensities below CF. Two examples of this are shown in the response maps in Fig. 8. The firing rate at high intensities also tended to be greater than that seen for comparable intensities at CF (Fig. 8). This increase in firing rate resulted from an increase in neuron discharge during the sustained portion of the response to tones (Fig. 5B). Figure 9 shows examples of PST histograms for three neurons when tones were presented at or below CF. At comparable high intensities, the tendency for firing during the sustained portion of the response to tones was highest when tones were presented below CF (Fig. 9).

Tones presented at the high-frequency edge of a neuron’s response area evoked an onset and an occasional offset action potential (Fig. 10). Presentation of such tones also resulted in a drop in the level of the sustained depolarizing component (Fig. 10A). At intensities sufficient to produce action potential generation during the initial depolarizing component, the membrane potential fell below resting during the sustained component (Fig. 10B). Long-lasting hyperpolarization was also observed in some neurons in the absence of spike generation (Fig. 10A).

Long-lasting hyperpolarization was also seen in neurons during the presentation of frequency tones just above the high-frequency edge of their response area (Fig. 11). In Fig. 11, the neural response to a 7-kHz tone (2 kHz above the neurons’ action potential response limit) and a CF tone (3.6 kHz) is depicted together with the neural response of this cell to the two tones presented in unison. Hyperpolarization was seen during the presentation of the 7-kHz tone (Fig. 11A). When the CF tone and the 7-kHz tone were presented in unison, a drop in the level of depolarization was seen when compared with the presentation of the CF tone alone (Fig. 11B). This drop in depolarization resulted in a decrease in action potential generation (Fig. 11C). This neuron also showed a hyperpolarization after the cessation of the CF tone, which was typical in O$_C$ neurons showing medium to high levels of tone-evoked sustained activity (Fig. 11B).

The long-lasting hyperpolarizing response to tones presented
at and above the high-frequency edge of a neuron’s response area decreased with more negative membrane potential (Fig. 12), reversing close to the chloride equilibrium potential of $\pm 75$ mV (McCormick 1998).

Response to click stimuli

In response to click stimuli, a single depolarizing component was evident that was similar to the initial depolarization evoked by acoustic tone stimulation. This depolarizing component was followed by a long-lasting hyperpolarization (Fig. 13A). With increasing click intensity both the depolarization and hyperpolarization increased in amplitude (Fig. 13, A–D). At spike threshold a single action potential was evoked (Fig. 13E) followed by a short-duration hyperpolarization. At just above spike threshold, both the short-duration hyperpolarization and the longer-lasting hyperpolarization were evident. As the intensity increased, the number of action potentials increased as did the amplitude of the longer-lasting hyperpolarization (Fig. 13, F and G). This long-lasting hyperpolarization was not seen after spontaneous action potentials (Fig. 13H).

The ability of clicks to evoke action potentials on successive presentations decreased as the interclick interval was reduced (Fig. 14). Summation of depolarizing potentials became evident at interclick intervals of 4 ms (Fig. 14B). Clicks presented 2 ms apart resulted in an initial onset spike and sustained depolarization with small depolarizing potentials present on every click presentation (Fig. 14C).

Response to intracellular current injection

The response to intracellular current injected through the microelectrode (from $-0.6$ to $+0.8$ nA) was recorded in two cells. Both cells showed similar responses to current injection. An example of this response is shown for one cell in Fig. 15. These neurons displayed action potential firing throughout the duration of a 500-ms depolarizing pulse (Fig. 15, A and B) and showed linear current–voltage relationships over a wide range
Action potentials were followed by a short-duration hyperpolarization (relative to current-induced depolarizing levels; Fig. 15A). High rates of firing were seen at high depolarizing levels in one neuron followed on cessation of the depolarizing pulse with long-lasting hyperpolarization (Fig. 15A).

Morphological characteristics

Of the 15 O_C neurons, 7 were identified morphologically as large multipolar stellate neurons in the VCN (Fig. 16). No morphological or physiological differences were observed between neurons located in the anteroverentral cochlear nucleus and those in the posteroverentral cochlear nucleus. Neurons showed extensive arborization of branching dendrites. They had large cell bodies (20- to 30-μm diam) and an axon, which in one case could be traced dorsally toward the dorsal acoustic stria with collateral branches extending within the confines of the nucleus (Fig. 16D).

Discussion

This investigation demonstrated that O_C neurons show dual-component processing capabilities. They show an intensity-dependent initial component from which the onset spikes are generated. This is followed by a sustained component occurring at lower depolarization amplitude that is dependent on both the frequency and intensity of the acoustic presentation.

The tone-evoked pattern of response of these O_C neurons may also be analogous to that of transient chopper (C_t) neurons. Rhode and Smith (1986), in their figure caption describing typical tone-evoked response patterns in the cochlear nucleus, state that O_C units are sometimes called C_t units when the late activity is vigorous. Smith and Rhode (1989) refer to the intracellular studies of Romand (1978, 1979) that relate to C_t units. They state that the descriptions of these units are similar to those observed in their O_C neurons. In this investigation we classified our units as O_C in accordance with Rhode and Smith (1986). Although others established various classification methods to distinguish between O_C and C_t units (Blackburn and Sachs 1989; Winter and Palmer 1995), absolute criteria for this distinction are absent. In the absence of absolute criteria it is possible that some of our neurons may also be classified as C_t neurons by others. However, as the neurons recorded in this investigation show similar intracellular response features to tones and share similar morphology, it is likely that they represent a uniform cell class.

Intensity and frequency coding with dual-component processing

O_C neurons are unique in that they respond over a wide frequency and intensity range (Rhode and Smith 1986). In four
cells reported in this investigation with CFs of ~10 kHz, the mean $Q_{10}$ values for these neurons was 3.1, which is similar to the mean $Q_{10}$ value reported by Jiang et al. (1996) at this frequency for onset neurons in guinea pigs. Jiang et al. (1996) report that this value is significantly lower than auditory nerve $Q_{10}$ values at comparable CFs. This ability to respond over a wide frequency range together with the dual-component depolarization present in $O_C$ neurons may make it possible for them to code frequency in addition to intensity information. The initial component appears to be intensity dependent. At CF, the amplitude of the initial component increases in amplitude with increasing tone intensity, leading to action potential generation. After initiation of the onset spike one to four additional spikes emerge from the initial depolarizing component with further increases in intensity. This pattern is repeated when frequencies are presented below CF, although the intensity required for depolarization and action potential generation is increased. This is not the case when tones are presented at frequencies above CF, where little change in the amplitude of the depolarization is seen with increasing intensity. These results suggest that intensity is coded by the initial component only when frequencies are presented below or close to CF.

The sustained component depends on both the frequency and intensity of acoustic presentation. During the presentation of tones the amplitude of the sustained depolarization increased with increasing intensity. However, this increase was limited by the frequency of the tone presented. The change in depolarization amplitude was greatest at the low-frequency edge of a neuron's response area and decreased with increasing frequency.

Our results also suggest that further information about the frequency of the tone may also be provided by the transition between the initial and sustained components. Frequency alone appears to determine the time required for the depolarization to decrease from its maximum during the initial component to the level of the sustained depolarization. Thus the initial component, the transition time, and the sustained component reflect elements of the acoustic stimulus. These changes in membrane depolarization may play a role in the coding of frequency and intensity information contained within complex stimuli.

FIG. 11. Inhibitory effect on an $O_C$ neuron when tones are presented just above the high-frequency edge of its response area (~5 kHz). A: intracellular response to tones presented at 7 kHz (shown at 80 dB SPL; CF of neuron ~3.6 kHz) evoked depolarization. A, left: the intracellular response to a single 50-ms, 7-kHz tone is shown. Synaptic noise was present throughout the trace. The average intracellular response over 15 stimulus presentations (A, right) shows the hyperpolarization persisting beyond the cessation of the tone. B: intracellular response to a single presentation of a 50-ms CF tone is shown (B, top left; 45 dB SPL) together with the intracellular response seen on presentation of the CF and the 7-kHz tone (B, top right). The average intracellular response over 20 presentations of the single tone and the 2-tone combination is shown below each trace. The tone presentation protocol is indicated at the bottom. Note the presence of hyperpolarization after cessation of tones in both protocols and the drop in the level of depolarization when both tones are presented in combination (dashed line and dotted line indicate sustained level seen during the presentation of the combined tone and resting potential, respectively). C: PST histograms showing spike output over 50 repetitions to the CF tone (left) and the CF tone followed by the 7-kHz tone (right). Note the drop in neural firing during the presentation of the combined tone. Protocol in C as in B; the CF and 7-kHz tones were presented over 50 ms at 45 and 80 dB SPL, respectively.

FIG. 12. Effect of membrane potential changes on the hyperpolarization evoked by a 7-kHz (70 ms) tone presented to the same neuron whose responses are depicted in Fig. 11. A–D: hyperpolarization decreased in amplitude the more negative the holding potential, reversing close to the mammalian chloride ion equilibrium potential (approximately ~75 mV) (McCormick 1998). Dotted line indicates the holding potential.
Neural mechanisms of dual-component processing

The results obtained in this investigation also provide clues to the neural mechanisms underlying dual-component processing by O\textsubscript{C} neurons. The short latency of the initial depolarizing component suggests that it is a response to auditory nerve fibers emanating from spiral ganglion cell axons. The wide frequency range over which these O\textsubscript{C} neurons are responsive suggests that these neurons receive extensive convergence of auditory nerve fibers. The O\textsubscript{C} response type was identified in the VCN as multipolar cells receiving the bulk of their innervation onto the cell soma (Smith and Rhode 1989). This innervation pattern may account for the low variability seen in first spike latency. Palmer et al. (1996) have shown that coincident detection of inputs across a range of CFs may be required to produce action potential generation in these neurons. The low spike variability may be attributed to coincident arrival of this converging excitatory auditory nerve input.

The drop to sustained levels of depolarization after the initial depolarizing component may result from the presence of a voltage-gated conductance intrinsic to the neuron itself or from polysynaptic inhibitory drive. Smith and Rhode (1989) discuss the possibility that a voltage- or calcium-activated potassium conductance (\(I_{K,Ca}\)) may produce a dip in the level of depolarization, resulting in a drop in spike activity. Two \(I_{K,Ca}\) currents were identified in neurons, \(I_{C}\) and \(I_{AHP}\) (Adams and Galvan 1986; McCormick 1998). \(I_{C}\) is rapidly inactivated and helps to maintain the frequency of action potential generation by causing marked hyperpolarization after the occurrence of each spike (McCormick 1998). \(I_{AHP}\) is slowly inactivated and is seen after a number of action potentials as prolonged afterhyperpolarization (McCormick 1998). Both these currents appear to play a part in the O\textsubscript{C}-type response. A rapid hyperpolarization consistent with \(I_{C}\) currents was seen after spontaneous click- and tone-evoked action potentials. Injection of depolarizing current also evoked action potentials with short-duration afterpotentials that fell below the current-induced membrane potential. Evidence for the longer-lasting \(I_{AHP}\) current can also be seen in our intracellular records. After the cessation of depolarizing current injection a long-lasting hyperpolarization was seen. Also, in neurons showing high levels of tone-evoked sustained activity, a long-lasting hyperpolarization was present after cessation of the tone. This long-lasting hyperpolarization may be attributed to an \(I_{AHP}\) current and may play a role in spike frequency adaptation.

However, because there is a large sustained depolarization at the low-frequency edge of a neuron’s response area (e.g., 3 kHz, Fig. 5), it is unlikely that its lesser amplitude at higher frequencies is due to the activation of a voltage-gated potassium conductance but rather may result from recruitment of inhibitory postsynaptic potentials (IPSPs). Furthermore, when tones were presented at or just above the high-frequency edge of a neuron’s response area, hyperpolarization of the membrane was evident. This hyperpolarization had a reversal potential more positive than that for potassium ions and close to the chloride equilibrium potential of \(-75\) mV (McCormick 1998), suggesting a possible IPSP response. Smith and Rhode (1989) discuss the possibility that inhibition may also contribute to the drop in spike activity observed after the initial chopping spikes. Inhibition was also proposed to be involved in shaping the C\textsubscript{T}-response type (Banks and Sachs 1992; Blackburn and Sachs 1989, 1992).

Evidence for inhibition on O\textsubscript{C} neurons can also be seen with click stimuli. Hyperpolarization was observed in this investigation in the presence or absence of action potentials evoked by a single click stimulus. Click stimulation would excite not only a large proportion of excitatory projections to O\textsubscript{C} neurons but would also evoke polysynaptic inhibitory and excitatory drive from intrinsic or extrinsic sources. The resultant hyperpolarization may be due to interplay between such excitatory and inhibitory influences. On presentation of successive click stimuli, hyperpolarization was not evident, although action potential generation decreased, suggesting the presence of inhibitory influences.

However, in past in vivo intracellular investigations, inhibition was not consistently seen. Smith and Rhode (1989) rarely observed dips in the level of depolarization or distinct hyperpolarization below the cell’s resting potential that might indi-
cate inhibition, whereas Feng et al. (1994) did observe hyperpolarizing influences in a Cf neuron akin to those observed in this investigation. This inconsistency may in part be related to the frequency of presentation as hyperpolarizing responses below resting potential were only seen in this investigation when tones were presented at or above the high-frequency edge of the neurons’ response area. The use of a nonbarbiturate anesthetic may also account for the presence of hyperpolarization seen in this investigation. The ability of barbiturates to influence inhibitory mechanisms was well documented by Evans and Nelson (1973) in the cochlear nucleus. In this investigation, we used urethan, which when compared with barbiturates appears to have only minor effects on synaptic transmission (Crawford 1970; Maggi and Meli 1986).

Further evidence for the involvement of inhibitory mechanisms in shaping the Oc response is provided by Ferragamo et al. (1998). They demonstrated with an in vitro preparation that D-stellate cells receive both glycinergic and GABAergic inhibition. D-stellate cells responded to auditory nerve stimulation with a fast followed by a slow depolarization. Although there was an absence of hyperpolarization, application of picrotoxin, a GABA_A receptor blocker, to the cochlear nucleus slice enhanced the firing of D-stellate cells. Ferragamo et al. (1998) propose that this GABAergic inhibition may originate from Golgi cells in the superficial granule cell domain. This response to auditory nerve stimulation is also seen in a stellate cell response type to intracochlear electrical stimulation recorded in vivo (Paolini and Clark 1998). This response type also showed little visible evidence of inhibition, although a

FIG. 14. Intracellular responses to successive click stimuli presented at 100 (A), 250 (B), and 500 (C) Hz. Vertical bars under trace indicate occurrence of an auditory click presented at 80 dB SPL.

FIG. 15. Intracellular responses to current injection of the Oc neuron whose responses to tones are depicted in Figs. 11 and 12. Ai: in response to a +0.5-nA, 500-ms depolarizing current pulse the neuron responded with action potentials throughout its duration. Aii: these action potentials were followed by a short-duration hyperpolarization relative to current-evoked membrane potential (*). On cessation of the depolarizing pulse, a long-lasting hyperpolarization was present (dotted line indicates resting potential). B: response to a series of hyperpolarizing and depolarizing pulses (-0.5 to +0.3 nA, in steps of 0.2 nA) evoked stepwise membrane potential changes. These changes are reflected in the current-voltage curve (C). Because of the presence of synaptic noise, the current-induced membrane potential level was measured with 10-point averaging of traces sampled at 10 kHz. Action potentials were truncated for clarity (spike height ~60 mV). Stimulus protocol is indicated at bottom of intracellular traces.
spike could not be evoked on the second excitatory postsynaptic potential response. Results obtained in this investigation and those of previous investigations (Ferragamo et al. 1998; Paolini and Clark 1998) suggest that polysynaptic inhibitory drive may play an important role in regulating the influence of excitation on these OC neurons.

In addition to GABAergic inhibition, glycinergic inhibitory input may also result from tuberculoventral cells in the dorsal cochlear nucleus (DCN) (Saint Marie et al. 1991; Wickesberg and Oertel 1990). Our recent study has shown that the DCN is an important contributor to on-frequency inhibition of VCN activity (Paolini et al. 1998). Suppression of DCN by application of muscimol, a GABA agonist, resulted in a decrease in response threshold for units in the VCN (Paolini et al. 1998). Extrinsic inhibitory input may also originate from the superior olivary complex, which has been shown to be an important source of inhibitory input to the cochlear nucleus (Ostapoff et al. 1997; Saint Marie et al. 1993).

Inhibitory projections may contribute to the OC response through shunting inhibition, as proposed by Smith and Rhode (1989). They suggest that the output of the cell may be reduced when the membrane potential is close to the reversal potential of chloride ions. At this potential, the amplitude of any depolarization will be substantially reduced when the chloride channel is activated, leading to increased conductance. Shunting inhibition may account for the drop in the level of depolarization observed during the sustained component. This decrease in the level of depolarization observed during the sustained component may result from a decrease of inhibitory drive or of shunting inhibition.

Alternatively, the increase in the level of sustained depolarization seen on presentation of tones on the low-frequency side of CF may also be explained by an increase in auditory nerve fiber convergence. As the frequency is decreased and at high intensities more auditory nerve fibers are recruited as low-frequency tails are commonly seen in the tuning characteristics of these fibers. However, an increase in the level of depolarization and spike activity was not seen during the initial component of the response when tones were presented at these frequencies. This suggests that, although more auditory nerve fibers are excited by low-frequency stimuli at high intensities, it is unlikely that they all contribute to the summation of inputs to OC neurons.

Proposed model

A model can be put forward to explain the changes in OC membrane responses to tones seen in this investigation. If we consider inhibition to be primarily from tuberculoventral cells, inhibitory influences would be expected to diminish the further away the frequency is presented below CF, as inhibitory projections are arranged in frequency-specific tonotopic manner (Wickesberg and Oertel 1988). However, as OC neurons are responsive to a large frequency range, excitation from auditory nerve input would still be evident at frequencies further away from CF. The
increase in amplitude of the sustained component at frequencies away from CF may be a result of a drop in the inhibition from these tubuloventral cells as the frequency presented begins to fall outside their response area. O_c neurons then may receive both excitation from auditory nerve and perhaps feedforward inhibition from tubuloventral neurons.

This presumed feedforward inhibition would appear to be strongest at frequencies above CF. This offset in the tonotopic extent of inhibition suggests that O_c-type neurons may also receive inhibitory input from local stellate cells within the VCN. Wickersberg and Oertel (1988) observed that the stellate cells labeled in the VCN after local injection of HRP were located only dorsal to the injection site, indicating that intrinsic projections relay information only from high- to low-frequency areas. If we assume these cells to be inhibitory, then the extent of inhibition would be greatest when higher frequencies are presented. This is reflected in our data, where onset neurons show a decrease in the level of sustained activation at frequencies above CF.

At these higher frequencies a depolarizing or action potential is often seen on cessation of the acoustic tone. An anode break effect could be responsible for the off response, although no offset action potential was seen in the two cells tested when hyperpolarizing current was injected intracellularly. Another possible explanation is that, as the tone is turned off inhibition is removed, resulting in rebound excitation caused by subsequent reassertion of tonic excitatory influences. Tonic excitation may arise from auditory nerve fibers that have been shown to contain predominantly low to medium levels of spontaneous activity (Liberman 1991).

Implications for sound processing

This investigation highlighted an important role of O_c neurons. They are able to vary their response characteristics depending on the frequency and intensity presented. Most of this processing may be achieved by interaction of excitatory and inhibitory influences. The influence of inhibition varies depending on the frequency presented. The role of this inhibition may not be to encode frequency information in the presence of background noise, as lateral suppressive sidebands are not observed in O_c neurons (Rhode and Greenberg 1994). O_c neurons may be particularly adept at processing both the frequency and intensity of sound. O_c neurons are most responsive during the initial component at CF, with maximum sustained depolarization seen at frequencies below CF at high intensities.

In conclusion, these neurons may not only be responsible for detecting the onset of acoustic stimuli but may also be responsible for processing frequency and intensity features contained within an acoustic stimulus. The initial component is consistent with auditory nerve excitation, whereas the drop to sustained levels seen after the initial excitation may be a result of feedforward or shunting inhibition that predominates above CF. The interplay between excitatory and inhibitory influences may underlie the processing by these O_c neurons.

We thank R. K. Shepherd and J. S. McKenzie for comments and suggestions regarding this manuscript and R. E. Millard for technical support.

This work was funded by The Hearing Research Foundation of The Bionic Ear Institute.

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Received 18 September 1998; accepted in final form 21 January 1999.

REFERENCES


