Comparison of Responses of Neurons in the Mouse Inferior Colliculus to Current Injections, Tones of Different Durations, and Sinusoidal Amplitude-Modulated Tones

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Tan ML, Borst JG. Comparison of responses of neurons in the mouse inferior colliculus to current injections, tones of different durations, and sinusoidal amplitude-modulated tones. J Neurophysiol 98: 454–466, 2007. First published May 16, 2007; doi:10.1152/jn.00174.2007. We made in vivo whole cell patch-clamp recordings from the inferior colliculus of young-adult, anesthetized C57/B16 mice to compare the responses to constant-current injections with the responses to tones of different duration or to sinusoidal amplitude-modulated (SAM) tones. We observed that voltage-dependent ion channels contributed in several ways to the response to tones. A sustained response to long tones was observed only in cells showing little accommodation during current injection. Cells showing burst-onset firing during current injection showed a small response to SAM tones, whereas burst-sustained cells showed a good response to SAM tones. The hyperpolarization-activated nonselective cation channel $I_h$ had a special role in shaping the responses: $I_h$ was associated with an increased excitability, with chopper and pauser responses, and with an afterhyperpolarization following tones. Synaptic properties were more important in determining the responses to tones of different durations. A short-latency inhibitory response appeared to contribute to the long-pass response in some cells and short-pass and band-pass neurons were characterized by their slow recovery from synaptic adaptation. Cells that recovered slowly from synaptic adaptation showed a relatively small response to SAM tones. Our results show an important role for both intrinsic membrane properties—most notably the presence of $I_h$ and the extent of accommodation—and synaptic adaptation in shaping the response to tones in the inferior colliculus.

INTRODUCTION

The inferior colliculus (IC) receives ascending afferent information from almost all of the major auditory nuclei in the lower brain stem. Auditory information from these nuclei is analyzed and transformed by the cells in the IC and relayed to the thalamus and the cortex. The relation between the specific complement of ion channels and the auditory role of IC neurons is still largely unexplored (Oliver 2005). In response to DC injections in intracellular or whole cell recordings, the cells in the IC can respond with a number of distinct firing patterns (Bal et al. 2002; Koch and Grothe 2003; Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001; Tan et al. 2007). Four important features in their response to current injection are the extent of accommodation during current injection, the presence of burst firing, the presence of an $A$-type potassium current leading to buildup-pauser firing, and the presence of the hyperpolarization-activated cation channel ($I_h$). The significance of these features for auditory processing in the IC is not yet known, but, for example, $I_h$ has been predicted to be involved in the afterhyperpolarization (AHP) following bursts of action potentials, in generating onset responses and in synaptic integration (Koch and Grothe 2003).

IC neurons vary not only in their response to current injection, but sound-evoked responses, as measured in extracellular recordings, also vary widely between cells (Chen 1997; Ehrlich et al. 1997; Le Beau et al. 1996; Palombi and Caspary 1996; Rees et al. 1997; Willott and Urban 1978; Xia et al. 2000). It is not yet known whether the situation in the IC is similar to the cochlear nucleus, where the relation between the firing patterns during current injection, the specific inputs, and output projections and the auditory role is relatively stereotypical (Cant 1992; Rhode and Greenberg 1992). To explore this relation, we compared the response to tones of different durations and to sinusoidal amplitude-modulated (SAM) tones with the response to current injection during in vivo patch-clamp recordings from the IC (Casseday et al. 1994) of young-adult mice. We chose tones of different durations and SAM tones as a stimulus because they have been extensively studied using extracellular recordings (Brand et al. 2000; Casseday et al. 1994; Chen 1998; Pérez-González et al. 2006; Pinheiro et al. 1991; Potter 1965; Xia et al. 2000), but the cellular mechanisms that allow cells to respond specifically to the duration of a sound and the mechanisms that allow synchronization of neural responses to the envelope of SAM tones (Krishna and Semple 2000; Langner 1983; Langner and Schreiner 1988; Rees and Møller 1983) are largely unknown.

METHODS

Animal procedures were in accordance with guidelines provided by the animal committee of the Erasmus MC. Patch-clamp recordings from the IC of young-adult, anesthetized C57/B16 mice were made exactly as described in our companion paper (Tan et al. 2007). Average ABR (auditory nerve–brain stem evoked response) thresholds for 1- to 2-mo-old C57/B16 mice range from 15 to 25 dB SPL in the 6- to 32-kHz range (Hunter and Willott 1987).

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Auditory stimulation

Sound stimuli were presented in closed field: speaker probes were inserted into both ear canals and stabilized with silicone elastomeres (World Precision Instruments, Sarasota, FL). Auditory stimuli were generated with Tucker Davis Technologies hardware (TDT, System 3, RP2.1 processor, PA5.1 attenuator, ED1 electrostatic driver, EC1 electrostatic speaker). Stimulus generation was controlled by a custom-made program written in MATLAB (version 7.0.4; The MathWorks, Natick, MA). All experiments were performed in a single-walled sound-attenuated chamber (Gretch-Ken Industries; attenuation ≥40 dB at 4–32 kHz).

DETERMINATION OF THE CHARACTERISTIC FREQUENCY. Pure tones (1–64 kHz in five steps between octaves) were presented at the contralateral ear starting at 80 dB SPL with decreasing steps of intensity (5 dB per step). Tones had durations of 50 ms and a 2-ms rise time, repeated ≥20 times at an interval of ≥150 ms. The frequency with the lowest intensity that elicited an excitatory postsynaptic potential (EPSP) of ≥1 mV was identified as the characteristic frequency (CFEPSP). This definition is different from that used in extracellular recordings, where CF is the frequency with the lowest intensity for eliciting an action potential.

DURATION TUNING. Tones of different durations at the characteristic frequency were presented in a fixed order. Durations increased from 1, 2, 4, to 512 ms with 0.5-ms rise time and an interval of 150 ms between the end of a tone and beginning of the next tone. This set of different durations was repeated ≥20 times with 250 ms between sets. Their amplitude was typically 40 dB above the EPSP threshold.

AMPLITUDE MODULATION. To study AM, 200-ms tones at the characteristic frequency were 70% sinusoidally modulated in amplitude according to

\[ s(t) = [1 + 0.7 \sin(2\pi f_m t) \sin(2\pi f_0 t)] \]

where \( s(t) \) is the waveform of a tone at the characteristic frequency \( f_0 \) that was modulated at a lower frequency \( f_m \), which ranged between 10 and 640 Hz. The tone included a 2-ms rise time, independent of modulation frequency. These sinusoidal amplitude-modulated (SAM) tones were repeated 20 times at an interval of 150 ms.

CALIBRATION. Sound intensities were calibrated between 0.5 and 65 kHz with a condenser microphone (ACO Pacific type 7017, MA3 stereo microphone amplifier, TDT SigCal software). During calibration we used a 3-mm-diameter PVC heat-shrinking tube to mimic a rodent external auditory ear canal. Its diameter and shape were based on the reconstruction of four young-adult rat external auditory ear canals. The speaker probe was inserted at one end of the tube at a distance of 4 mm to the diaphragm of the microphone, resembling the distance from tragus to ear drum.

Analysis

GENERAL. Analysis was done with custom-written programs that used the NeuroMatic environment (version 1.91, kindly provided by Dr. J. Rothman, University College London) within Igor Pro 5 (WaveMetrics, Lake Oswego, OR). Amplitudes, 20–80% rise times, and delays were measured after spike truncation. Spikes were truncated by interpolating between the membrane potential 1–2 ms before and after the peak of the action potential. Unless noted otherwise, rise times and delays refer to the EPSP evoked by the last tone in the duration protocol. At least 20 traces were averaged. Peak amplitudes were corrected for noise by subtraction of the SD of the resting membrane potential, measured for a period of ≥10 ms before sound onset. Delays were measured as the delay between sound onset and the intersection between a (horizontal) line through the average baseline and a line through the points of the rising phase at which the EPSP was 20 and 80% of maximal.

Data on the activation of \( I_{h} \), the classification of different firing types, the accommodation index (AI), and membrane properties (such as input resistance, membrane time constant, resting membrane potential, and spike threshold) were taken from the companion paper (Tan et al. 2007). AHPs had to be ≥1 mV. For inhibitory postsynaptic potential (IPSP) amplitudes the maximal value following any of the 10 different durations was used.

DURATION TUNING. Neurons were classified for duration tuning according to Brand et al. (2000). For neurons that regularly responded with spikes to tones, it was tested whether the spike count dropped to <50% of the maximum spike count for any duration. If the drop was for long durations, the neuron was classified as short-pass; conversely, it was classified as long-pass. If the spike count was <50% of the maximum count both for short and for long durations, the neuron was classified as band-pass. For neurons that infrequently or never responded with spikes, the size of the EPSPs (after truncation of spikes if present) was used for the classification. Analogously to the spike count criterion, a 50% reduction of EPSP size was used to classify neurons as short-pass, long-pass, or band-pass. Neurons that did not match the criteria for either short-pass, long-pass, or band-pass were classified as untuned. The responses to 512-ms auditory stimulation were further analyzed. Cells that fired action potentials in response to tones were identified as onset when they fired at most a few action potentials at the onset, sustained when they fired throughout the tone, or pauser when a distinct firing pause could be recognized after the onset of the stimulus (Le Beau et al. 1996; Rees et al. 1997; Willott and Urban 1978).

SAM TONES. The modulation amplitude was calculated in two different ways: 1) by fitting a sine wave to the EPSP amplitudes with a period identical to the period of the sound modulation and 2) by averaging every period of the membrane potential and calculating the peak-to-peak amplitude. To avoid the spurious effects of an onset response, the same time period was used for both approaches, which was typically the last 100 ms of the tone. Occasionally, shorter time periods were used when the average membrane potential did not reach a plateau value. Both methods are compared in Fig. 7A. Unless stated otherwise, the amplitude method has been used in the presentation of the results.

VECTOR STRENGTH. The vector strength \( R \), sometimes called the synchronization index, was calculated as follows (Goldberg and Brown 1969)

\[ R = \left( \frac{\sum \cos \theta + \sum \sin \theta}{n} \right) \]

where \( \theta \) is the phase (between 0 and 2\( \pi \)) of the spike time \( i \) modulo the modulation period. Because this vector is normalized to the total number of spikes \( n \), it takes on values between 0 and 1, with a value of 0 indicating no phase locking and 1 perfect phase locking. Only spikes that occurred >100 ms after the tone onset were included, to avoid inclusion of cells with an onset response that had high vector strengths. Our definition thereby deviates from other studies, in which the onset response was partially or fully included (e.g., Walton et al. 2002), but in which case the rise time of the tones was dependent on the modulation frequency. It was not necessary to correct for spontaneous firing because this was generally quite infrequent.

STATISTICS. Data are presented as means ± SE. A difference in the mean of two groups was assessed by Student’s t-test. Differences in the mean of more than two groups were assessed by ANOVA, followed by a Tukey’s HSD test. Statistical significance of \( R \) was evaluated using the Rayleigh test (Mardia and Jupp 2000). Values of
P < 0.05 were judged as statistically significant, unless noted otherwise.

RESULTS

In total, 45 cells from the mouse inferior colliculus (IC) were recorded in whole cell current clamp. These cells are a subset of a larger number of cells whose passive properties and response to current injection were described in the companion paper (Tan et al. 2007). They had a characteristic frequency (CF of EPSP) of 22.0 ± 1.4 kHz (mean ± SE; range 5–49 kHz) at a threshold of 29 ± 2.7 dB (range 0–80 dB).

Spiking versus nonspiking

We tested the response to tones of increasing duration at CF of EPSP, using tones ranging from 1 to 512 ms, given at an intertone interval of 150 ms. In all cells an EPSP was evoked in response to a long tone. The EPSP started 10.3 ± 0.7 ms after the onset of the tone and its 20–80% rise time was 8.3 ± 1.5 ms. In 30 of 45 cells the EPSPs were sufficiently large to trigger spikes; in the other cases, only subthreshold responses were observed. In the cells in which action potentials were reliably triggered (i.e., in more than half of the tone presentations) in response to the 512-ms tone, the median spike delay was 10.3 ms after tone onset (n = 17; range 7.9–181 ms). The median delay between the onset of the EPSP and the first spike was 1.8 ms (range 0.9–172 ms) in these cells. In addition, six of the 45 cells showed spikelets. In two of these, the spikelets could be triggered by sound.

Tone-evoked spikes versus spikes evoked by constant-current injection

Of the 30 cells in which the tones triggered spikes, 17 cells showed an onset response, three a pauser response, and ten fired in a sustained manner during long tones. This response depends on both the synaptic input and on the membrane properties of the cell. To partly assess the contribution of the latter, we compared the responses to tones with the responses to constant-current injection. During constant-current injection, nine of the 45 cells responded as sustained, 15 as accommodating, seven as buildup-pauser, six as accelerating, three as burst-onset, and five as burst-sustained. As described in our companion paper (Tan et al. 2007) we use the term “accommodation” instead of “adaptation” for current injections to avoid confusion with synaptic adaptation. The responses to current injection could be used to predict some aspects of the response to tones. For example, all burst-sustained cells responded both to depolarizing constant-current injections and to tones with bursts of action potentials. However, the relation was not always that straightforward. Figure 1 shows two different accelerating cells that both showed a nonaccommodating response to constant-current injection (Fig. 1, A and C). Their response to tones, however, was very different. One cell showed an onset response (Fig. 1B), whereas the other fired in a burst-sustained manner during the tone (Fig. 1D). Apparently, a nonaccommodating response during constant-current injection is not sufficient to yield a sustained response during long tones. Figure 1E shows in a raster plot a comparison of the response to a 1-s depolarizing current injection and the response to the first (of ≥20) presentation of a 512-ms tone. Four cells are not displayed because they did not spike during the 512-ms tone. Although the first response was not always representative for the overall response to tones, from this plot it is clear that the cells that showed an onset response following tones could show both a nonaccommodating and an accommodating response during current injection. To compare the two types of cells, we calculated the ratio of the amplitude of the EPSP at the end of the 512-ms tone and the maximum EPSP amplitude during the same tone (“steady-peak EPSP ratio”) as a measure for the persistency of the EPSP during long tones. This ratio was clearly larger in the cells that fired in a sustained manner (0.5 ± 0.09; n = 13) than in the cells that had an onset response (0.16 ± 0.05; n = 17; P < 0.01), in agreement with the two cells illustrated in Fig. 1, B and D (steady-peak EPSP ratio was 0.43 and 0.76, respectively). Evidently, cells that fired in a sustained manner must have had a sustained excitatory drive during the long tones. We compared the steady-peak EPSP ratio for the longest tone duration and the AI, which is a measure for the amount of accommodation during constant-current injection (Tan et al. 2007). This comparison showed that in addition to a sustained excitatory drive, a nonaccommodating response to current injection is also a prerequisite for a sustained response during long tones (Fig. 1F). Cells with a sustained response during long tones (steady-peak EPSP ratio >0.7) had a small AI, which was on average 2.7 ± 1.1% (n = 8). These results indicate that sustained firing during current injection is a necessary but not a sufficient condition for a sustained response during long tones.

INHIBITION. Because all models that deal with duration tuning emphasize the role of inhibition, we looked at whether there was evidence for synaptic inhibition in the IC neurons during tone stimulation at CF of EPSP. In 15 of the 45 cells a short-latency IPSP was observed. An example is shown in Fig. 5C. Its amplitude ranged from −0.3 to −4.8 mV (mean −1.3 ± 0.4 mV), from a resting membrane potential ranging from −47 to −67 mV. In two of the cells with a fast IPSP, this IPSP was long lasting (>50 ms) and during long tones an IPSP–EPSP–IPSP sequence was present. In the other cells, either a sustained EPSP was present or the membrane potential returned to the baseline level. Delay of the EPSP in the cells with a fast IPSP was a bit longer (11.7 ± 1.1 ms, n = 15 vs. 9.7 ± 0.8 ms, n = 30), but this difference did not reach significance (P = 0.16). In seven cells that did not show an IPSP at CF of EPSP, short-latency IPSPs were observed at other frequencies (not shown). In addition, in 17 of the 45 neurons, following long (>8 ms) tones the membrane potential hyperpolarized. The amplitude of this AHP was −2.6 ± 0.2 mV (range −1.5 to −4.7 mV). As subsequently detailed, in 16 of these 17 cells the deactivation of the mixed cation current I_h during the tone contributed to or mediated this response.

Rebound spiking was not observed in response to auditory stimulation in any of the 45 cells.

OFF RESPONSES. In 10 of 45 cells we observed a depolarizing off-response after the tone. The average amplitude of this EPSP ranged from 0.4 to 3 mV (mean 1.5 ± 0.3 mV). The off-EPSPs were identified by their constant, short latency following the end of tones of different durations (Fig. 2). They
were unlikely to be a rebound response from an inhibitory input onto the same cell because in only two of the ten cells was there clear evidence for a late-IPSP during longer tones and in these cells the amplitude of the off-EPSP did not correlate with the size of the late-IPSP. Two other cells had an IPSP preceding the on-EPSP as well. In nine of ten cells the size of the off-EPSP was smaller than the size of the on-EPSP. In nine of ten cells the amplitude of the off-EPSP did not vary during long tones. The amplitude of the EPSP at the end of the tone was generally had relatively small-plateau amplitudes during long tones. The fifth cell showed a complex response with a depolarizing step (indicated below raster plot) that resulted in the response whose amplitude best matched the maximal excitatory postsynaptic potential (EPSP) amplitude elicited by the tone stimulation. Cells 1–12 had an onset-type response to sound, cells 13–23 a sustained, bursting response. Some cells responded with a short burst of two or more spikes during sound stimulation was similar, cells with evidence for depolarization was needed to trigger an action potential. As a result, although the maximal EPSP size that was reached during sound stimulation was similar, cells with evidence for $I_h$ were more likely to fire spikes in response to the tones.

Some cells responded with a short burst of two or more action potentials during a tone (Fig. 3, B and D). These bursts were often synchronized across trials, resulting in a chopper-like response (Fig. 3, C and E). In 12 of the 30 cells that spiked in response to the duration protocol, there was some degree of synchronicity, although the number of spikes was not always sufficient to assess this unambiguously. Of these 12 cells, 11 had evidence for the presence of $I_h$. In three of these cells, the depolarizing sag observed during hyperpolarizing current injections could be well described by a single-exponential function, with a time constant ranging from 12 to 18 ms, whereas in the other eight cells, two exponential functions were neces-
Summary of the comparison between current injections and tones. From the comparison between the response to sound-evoked stimuli and the response to constant-current injection we were able to show that the two were similar in many respects. Sustained spiking during tones was observed only when the neurons also showed sustained spiking during current injection. \( I_h \) was associated with chopper responses and pauser response and played an important role in the AHP following tones.

Next we investigated the type of duration tuning of the cells.

Duration tuning

Based on the criteria described in Methods, 19 of the 45 cells showed some form of duration tuning in response to the standard protocol in which tones of increasing duration were given in fixed order (Fig. 5; Table 2). The remaining 26 cells were classified as untuned.

Short-pass. Three cells showed a short-pass response because the response to brief tones was clearly larger than the sary. In these eight cells, a clear fast component was always observed, which averaged 71 ± 4% of the total contribution, with an average time constant of 44 ± 11 ms. The average membrane potential at the peak of the EPSP (after spike truncation) was −45.4 ± 1.6 mV (\( n = 12 \)) in the cells with a chopper-like response. Because the reversal potential of \( I_h \) lies between −29 and −40 mV in auditory neurons (Bal and Oertel 2000; Banks et al. 1993; Cuttle et al. 2001; Rodrigues and Oertel 2006), the deactivation of \( I_h \) during tones in these cells is expected to result in a hyperpolarizing sag.

Three of the chopper-like cells with a sustained component to the EPSP had a pauser response (Fig. 4, A and B). In these cells, the hyperpolarizing sag during longer tones was large enough to momentarily halt spiking. Only one of these three cells showed a fast IPSP. In addition, a similar hyperpolarizing sag was observed during constant-current injection (Fig. 4 C), arguing against a role for an inhibitory input in the pause.

\( I_h \) was associated with an AHP immediately following tones. Sixteen of the 17 cells that showed an AHP after tones >8 ms had clear evidence for the presence of \( I_h \). In the cells with evidence for \( I_h \), the amplitude and the time course of the AHP generally matched the AHP observed following a similar depolarization resulting from current injection. Conversely, all cells that had both \( I_h \) and a sustained EPSP showed an AHP (Figs. 3B and 4A). In the eight cells that had \( I_h \), but lacked an AHP, the sustained EPSPs were on average only 0.5 mV (range 0–1.9 mV), which was substantially lower than the sustained EPSPs in cells with \( I_h \) that did have an AHP (mean 7.4 mV, range 3–12.6 mV).

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**FIG. 2.** Off-EPSP. *Top 3 traces:* consecutive responses to a 32-ms (A) or 256-ms (B) 24.3-kHz (CFEPSP), 60-dB SPL pure tone. In the *top 2 traces* the small EPSP was able to trigger a spike at the end of the 256-ms tone. Onset EPSP was preceded by a small inhibitory postsynaptic potential (IPSP). *Bottom trace:* average of 20 responses after spike truncation, showing that the amplitude of the averaged off-EPSP was only 1 mV and that it immediately followed after the tone, for both the 32- and the 256-ms tones. Resting membrane potential was −53 mV.

**FIG. 3.** Chopper responses are associated with the presence of \( I_h \). A: response to 1-s constant-current injection of (from top to bottom) +150, +100, and −150 pA, as indicated below the traces. During hyperpolarizing current injection a depolarizing sag was observed. During depolarizing current injection a hyperpolarizing sag was observed, similar to the responses during tone presentations. B, top to bottom: responses to 3 consecutive 16-kHz (CFEPSP), 35-dB, 512-ms tone presentations, average trace (after spike truncation), and tone. Note the similarity of the time course of the hyperpolarizing sag with the sag during current injection (A). Vertical calibration bar is shown in D. C: peristimulus time histogram (PSTH) of spikes. Bin size 0.1 ms. D and E: same cell except horizontal scale is different to illustrate the chopper-like response to the onset of the last tone. Times in C and E are relative to tone onset.
response to longer tones (Fig. 5A). In all three cells a clear onset response was observed and in two of three cells the responses were suprathreshold. In one of the three cells a small, rapid IPSP preceded the EPSP and a small AHP followed the spikes. In the nonspiking cell a small excitatory off-response was observed. The response to current injection was very different from the response to tones, in that two of the three cells had a sustained response (accelerating and sustained).

**BAND-PASS.** In nine of the 45 cells, a band-pass response was observed. These cells preferentially responded to tones of certain durations and were less responsive to tones that were either briefer or longer in duration. As with the short-pass response, in all cases the responses were onset type. In seven of the nine cells spikes were evoked and in the other two the responses were subthreshold. One of these two cells is illustrated in Fig. 5B. In this cell, 1-ms tones evoked an EPSP with an average size of only 1 mV, which increased to around 10 mV at a duration of 32 ms. At longer durations, the EPSPs decreased again to <5 mV. Although the response at the end of the long tones was small, there was no direct evidence for a contribution of inhibitory conductances. EPSPs with sizes similar to those of spontaneous EPSPs were still observed at the end of long tones (e.g., response to 512 ms; Fig. 5B, top trace). The other cell with subthreshold responses showed a similar response. In the other seven cells with a band-pass response, responses were suprathreshold. In two cells there was clear evidence for synaptic inhibition. In both cells a fast IPSP preceded the EPSP. This IPSP outlasted the EPSP in one of these cells and, as a result, long tones resulted in a sustained hyperpolarization. In three of the five remaining band-pass cells, spikes were followed by an AHP. These cells all had evidence for the presence of \( I_h \) because they showed a depolarizing sag during hyperpolarizing current injection. None of the band-pass neurons showed an off-EPSP. The responses to current injection did not elicit a common response pattern in the band-pass cells. One cell had a buildup response, two accelerating, three accommodating, two burst-sustained, and one sustained.

**LONG-PASS.** In seven of the 45 cells we observed a long-pass response. In these cells the response increased with increasing sound duration. In six of the seven cells the EPSPs were sufficiently large to trigger spikes. Four of the long-pass cells had clear evidence for a fast IPSP, which was already observed at the shortest duration tested (1 ms). In three of these cells no EPSP could be observed for durations as long as 4–16 ms in the different experiments. In the fourth cell an EPSP already preceded the IPSP at short durations and, in addition, an off-EPSP was observed at longer durations. In these four cells it seems likely that the IPSPs contributed to the long-pass behavior of the cell. In the other three cells there was no evidence for the presence of a short-latency IPSP. In these cells, increasing tones evoked EPSPs of increasing size (Fig. 5C). Only one of the long-pass neurons showed an off-EPSP.

Both the rise times and the delays of the excitatory response to the longest tone differed significantly between the groups (Table 2). The clearest difference was that the rise times of the long-pass neurons were longer than those of the other neurons. This difference was mostly attributed to the cells with a fast IPSP, which had relatively large rise times (31.2 ± 7.1 ms, \( n = 4 \) vs. 9.0 ± 2.7 ms, \( n = 3 \)).

Of the seven long-pass cells, based on their responses to constant-current injection, two were classified as sustained, two as accommodating, two as buildup, and one as burst-sustained.

### Table 1. Properties of cells with and without hyperpolarization-activated current, \( I_h \)

<table>
<thead>
<tr>
<th></th>
<th>( I_h )</th>
<th>No ( I_h )</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_{in} ), MΩ</td>
<td>81 ± 6</td>
<td>99 ± 6</td>
<td>89 ± 5 (( P = 0.038 )^*)</td>
</tr>
<tr>
<td>( V_{m} ), mV</td>
<td>54.2 ± 1.0</td>
<td>−60.3 ± 1.5</td>
<td>57.1 ± 1.0 (( P = 0.002 )^*)</td>
</tr>
<tr>
<td>Absolute firing threshold, mV</td>
<td>−48.2 ± 0.8</td>
<td>−45.2 ± 1.2</td>
<td>−46.8 ± 0.7 (( P = 0.044 )^*)</td>
</tr>
<tr>
<td>Threshold-( V_{m} ), mV</td>
<td>6.0 ± 1.1</td>
<td>15.1 ± 1.9</td>
<td>10.3 ± 1.3 (( P &lt; 0.001 )^*)</td>
</tr>
<tr>
<td>EPSP(_{max} ), mV</td>
<td>8.0 ± 0.7</td>
<td>7.7 ± 0.9</td>
<td>7.9 ± 0.5 (( P = 0.8 )</td>
</tr>
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</table>

Values are means ± SE. \( R_{in} \), input resistance; \( V_{m} \), resting membrane; EPSP\(_{max} \), maximal EPSP size reached during sound stimulation. Asterisk (*) indicates differences between cells with and without evidence for the presence of \( I_h \) were significant (two-tailed \( t \)-test or \( \chi^2 \) test).

![Figure 4](http://jn.physiology.org/ Downloaded from)

**Fig. 4.** Pauser response and afterhyperpolarization (AHP) in a cell that has \( I_h \). A, top to bottom: 2 consecutive responses, average trace (after spike truncation), and 55-dB tone stimulus (CF\(_{peak} \) 16 kHz). Only the response to the last tone is shown. Traces were vertically offset for display purposes. Membrane potential was −51 mV. B, PSTH of spikes evoked by the last sound stimulus. Bin size 5 ms. C, response to 1-s constant-current injection of +50 pA (top trace) or −150 pA (bottom trace), as indicated below the traces. Traces are offset for display purposes. Note the similarity in the time course of the sags and the AHP with the hyperpolarizing sag and the AHP during tones (A).
Resting membrane potential, action potential threshold, input resistance, membrane time constant, the presence of $I_h$, and kinetics of $I_h$ did not differ significantly between long-pass, short-pass, band-pass, and untuned neurons.

**SUMMARY.** In summary, we observed four types of responses to tones of different durations, short-pass, band-pass, long-pass, and untuned. All four classes contained various types of firing patterns in response to current injection. In some, but not all, of the band-pass and the long-pass neurons, a contribution of a rapid-onset IPSP was apparent. The cells that showed a decrease in EPSP amplitude with the successive tones in the duration protocol in each case had an onset-type response; therefore preceding tones had to be responsible for this decrease. This means that for both the short-pass and the band-pass neurons synaptic adaptation played a crucial role.

**Response to SAM tones**

To further investigate the role of both membrane properties and short-term synaptic plasticity, we investigated the responses to sinusoidal amplitude-modulated (SAM) tones in a subset of 33 of the 45 cells. In these cells, 200-ms stimuli were given at the same carrier frequency as that for the duration stimuli, which was the characteristic frequency ($\text{CF}_{\text{EPSP}}$). The amplitude of these tones was 70% modulated at frequencies ranging from 10 to 640 Hz (Fig. 6). In many respects the response to the different SAM tones was similar to the response to the long, nonmodulated tones that were given in the duration protocol (Fig. 5). The large majority of cells responded with a short-latency EPSP, whose rise time and delay was generally independent of modulation frequency. The lack of a dependency of the initial EPSP of modulation frequency was ascribed to the design of the tones, given that the rise time of the SAM tones was always 2 ms, independent of modulation frequency. This onset-EPSP was followed by a plateau with an average amplitude that generally matched the amplitude of the plateau reached at the end of the last tone in the duration protocol. In addition, in many cells the membrane potential varied at the same frequency as the modulation frequency (Fig. 6).

This modulation did not arise from cross talk with the stimulation because it was not in phase with the SAM tone. The modulation was especially apparent for the lower frequencies between 20 and 80 Hz and the average of the EPSP modulation amplitudes at the same frequencies. Figure 7 shows that when the EPSP modulation amplitude is high, the
cells are more likely to phase lock their spikes to the SAM. However, the number of cells that showed robust spiking at all frequencies and the number of repetitions were too low to reliably assess the best-modulation frequency with respect to synchronicity.

In the six cells with the largest modulation amplitude we looked at the delay between stimulus and response. At 10 Hz, this delay was generally not very meaningful, in that the sine fit was often not good. Between 20 and 160 Hz the time delay between the SAM tone and the membrane potential fluctuations changed only slightly (Fig. 6G). In four of six cells it was a few milliseconds greater than the minimal delay, the delay between tone onset, and the start of the EPSP. In the other two cells, the sine-wave fits to the membrane potential fluctuations were not very good and the delays were less meaningful, although again there were no obvious changes in the delay between 20 and 160 Hz. For a modulation frequency of 160 Hz the delay was in each case already more than one sine-wave period.

Fig. 6. Response to sinusoidal amplitude-modulated (SAM) tones. A, top to bottom: example trace of membrane potential, average response (after spike truncation) of 20 stimuli, and SAM tone, which consisted of a 200-ms, 70-dB, 24.3-kHz tone that was 70% modulated at 20 Hz. Resting membrane potential was −67 mV. Same cell as displayed in Fig. 1, A and B. B: raster plot of spikes triggered by the SAM tone at a modulation frequency of 20 Hz. C: like A except modulation frequency of SAM tone was 320 Hz. Calibration bars also apply to A. D: raster plot of spikes triggered by the 320-Hz SAM tone. E: average of the response of the last two 20-Hz modulations after spike truncation. Fit with a 20-Hz sine wave is shown in gray. F: average response to last 125 ms of the 320-Hz tone, with the fit with a 320-Hz sine wave shown in gray. Note the difference in the scale with the traces in E. G, top panel: open circles show peak-to-peak amplitude of the averaged membrane potential as a function of frequency, closed circles the amplitude of the sine-wave fit to the averaged membrane potential. Membrane potential was averaged as described in E or F. Second panel from top: time delay (in milliseconds) between modulation of tone and of the fit with a sine wave of the same period to the data (filled squares). Broken line gives the average delay between tone onset and the start of the EPSP. Second panel from bottom: number of evoked spikes per sweep vs. modulation frequency (open triangles). Bottom panel: vector strength R vs. modulation frequency (diamonds). Filled diamonds indicate significant vector strength (Rayleigh test; P < 0.001).

Relation with responses during duration protocol. We also investigated to what extent the responses during the duration could predict the responses during the AM protocol. As a general measure for the ability of the cell to respond to SAM tones we used the average modulation amplitude in the range 20–80 Hz. The peak amplitudes of the response to the first tone (1-ms duration) of the duration protocol did not correlate with the SAM tone response (r = 0.01). As shown in Fig. 8A, cells that were classified as short-pass in the duration protocol had a relatively small SAM response, whereas cells that were classified as band-pass or long-pass in the duration protocol had a relatively large response to the SAM tones, compared with the response to a 1-ms tone. The poor response of the short-pass response to SAM tone could be explained by adaptation during the SAM tone because the short-pass cells all showed clear adaptation in the duration protocol. In contrast, the 1-ms tone is apparently a much less effective stimulus for the cells with a band-pass or a long-pass response in the duration protocol, which responded more effectively to longer...
For the 16-ms tone duration, the linear correlation coefficient to the duration protocol improved for increasing tone duration. The correlation between the response to SAM tones and across the individual cycles of the last 100 ms of each trace.

MODULATION. All four burst-sustained cells had average modulation amplitudes >1.5 mV (Fig. 8F). These four cells all showed clear burst firing during current injection, they all had \( I_h \) with a prominent rapid component, they all showed a chopper-like response during tones, and they all showed a vector strength of ≥0.5. The responses to unmodulated tones or to constant-current injection of one of these cells was shown in Fig. 3. Both during current injection and during tones, these cells showed burst firing on top of depolarization typically lasting on average between 30 and 110 ms in the different cells. These bursts were generally followed by a small AHP, which could be related to changes in the availability of \( I_h \). Apart from the four burst-sustained cells described earlier, there were also many cells with evidence for the presence of \( I_h \) that did not respond well to SAM tones. Of the other two cells with modulation amplitudes >1.5 mV, one was a buildup cell and the other one an accelerating cell, both with no evidence for \( I_h \). These results indicate that the presence of \( I_h \) is both not a

Fig. 7. Quantification of the response to SAM tones. A: relation between 2 methods to assess the amount of modulation for a modulation frequency of 20 Hz. Vertical axis gives the amplitude of the sine fit and the horizontal axis the peak-to-peak amplitude of the membrane potential, which had been averaged modulo the SAM tone period. Broken line indicates the identity relation. Insets show 2 examples. In the top left, the sine-wave fit, shown in gray, is very good. This example corresponds to the data point indicated as an asterisk. In the other one, shown in the bottom right corner, the fit is poor because of the presence of an IPSP. This point corresponds to the filled diamond. Vertical calibration bars: 0.5 mV; horizontal, 20 ms. B: relation between amplitude of the modulation and the modulation frequency. Amplitude of the modulation was the peak-to-peak amplitude of the averaged response to the SAM tone. For each modulation frequency, responses were averaged both across the traces and across the individual cycles of the last 100 ms of each trace. C: relation between vector strength and the average amplitude of the AM modulation at frequencies between 20 and 80 Hz. Vector strength was calculated as detailed in METHODS. Only cells that responded with one or more spikes during the last 150 ms of ≥2 of the 20 SAM tones of 20, 40, and 80 Hz were used. Solid line is the line fit (\( r = 0.66 \)).

The correlation between the response to SAM tones and to the duration protocol improved for increasing tone duration. For the 16-ms tone duration, the linear correlation coefficient \( r \) was 0.57. From Fig. 8B it is clear that the band-pass cells show a relatively poor response to the SAM tone response compared with the response to a 16-ms tone, whereas some of the long-pass cells responded more effectively to the SAM tones than to the 16-ms tone. The correlation coefficient still increased slightly for longer durations. The relation between the peak amplitudes of the response to the longest tone (512 ms) in the duration protocol and the SAM tone amplitudes is shown in Fig. 8C. At this point \( r = 0.64 \). It is again informative to look at the outliers. A band-pass cell responded better to the SAM tones than to the 512-ms tone, presumably because of the effects of adaptation during the duration protocol. The cells that responded relatively poorly to the SAM-tone protocol compared with the response to the 512-ms tone included a long-pass cell that responded very slowly with minimal spike delays >30 ms. Another group of cells that responded relatively poorly to the SAM tones (modulation amplitudes <1 mV) were characterized by low-plateau amplitudes (Fig. 8D). Several of these cells had off-responses, suggesting that the cells with off-responses were more specialized in detecting brief changes in sound intensity, rather than the more continuous waxing and waning on top of a steady tone that characterized the SAM tone.

Some cells were able to respond to high-modulation frequencies much better than others. This could be explained by 1) postsynaptic factors, such as the relative ability of the cells to follow rapid changes in conductance or 2) presynaptic factors, such as the presence of an input that is resistant to fatigue. To illustrate this ability to follow slow and rapid changes in membrane potential we compared the ratio of the modulation amplitude at 160 and at 20 Hz with the persistency of the response to the last tone of the duration protocol, for which the steady-peak EPSP ratio—the ratio between the amplitude at the end of the tone and the peak amplitude—was taken. Figure 8E shows that the two are clearly related (\( r = 0.61 \)). In contrast, the correlation between this modulation ratio and the time constant was much less obvious (\( r = -0.37 \); not shown).

There was also no obvious correlation of the SAM-tone response with age of the animal, its weight, recording depth, membrane resistance, membrane time constant, action potential threshold, characteristic frequency, sound level at threshold, the presence of spikelets, the presence of a fast IPSP, or the membrane potential.

RELATION BETWEEN FIRING PATTERN AND THE RESPONSE TO AM MODULATION. All four burst-sustained cells had average modulation amplitudes >1.5 mV (Fig. 8F). These four cells all showed clear burst firing during current injection, they all had \( I_h \) with a prominent rapid component, they all showed a chopper-like response during tones, and they all showed a vector strength of ≥0.5. The responses to unmodulated tones or to constant-current injection of one of these cells was shown in Fig. 3. Both during current injection and during tones, these cells showed burst firing on top of depolarization typically lasting on average between 30 and 110 ms in the different cells. These bursts were generally followed by a small AHP, which could be related to changes in the availability of \( I_h \). Apart from the four burst-sustained cells described earlier, there were also many cells with evidence for the presence of \( I_h \) that did not respond well to SAM tones. Of the other two cells with modulation amplitudes >1.5 mV, one was a buildup cell and the other one an accelerating cell, both with no evidence for \( I_h \). These results indicate that the presence of \( I_h \) is both not a
necessary and not a sufficient condition for a good phase-locking response.

SUMMARY AM TUNING. Cells with band-pass or long-pass duration tuning had a relatively large response to SAM tones, whereas short-pass cells did relatively poorly. Burst-sustained cells showed a relatively large response to SAM tones.

DISCUSSION

Although the response of IC neurons to current injections has been extensively characterized in slice recordings (Bal et al. 2002; Koch and Grothe 2003; Peruzzi et al. 2000; Sivaramakrishnan and Oliver 2001), the significance of the different firing patterns for auditory transduction has not yet been studied. In this paper we therefore compared the changes in the membrane potential of neurons in the mouse IC during constant-current injections with the responses to tones of different durations and to tones whose amplitudes were sinusoidally modulated. We observed that voltage-dependent ion channels contributed in several ways to the response to tones. For example, a sustained response to long tones was observed only in nonaccommodating cells. Moreover, the hyperpolarization-activated nonselective cation channel $I_h$ had a special role in shaping the responses: $I_h$ was associated with an increased excitability, with chopper and pauser responses, with an after-hyperpolarization following tones, and with a large response to SAM tones. Synaptic properties were more important in determining the responses to tones of different durations. A short-latency inhibitory response appeared to contribute to the long-pass response in some cells and short-pass and band-pass neurons were characterized by their slow recovery from synaptic adaptation. Cells that recovered slowly from synaptic adaptation showed a relatively small response to SAM tones.

Synaptic mechanisms in the response to SAM tones or tones of different duration

Our experiments provide the first characterization of the intracellular response of mouse IC neurons to tones of different duration and of SAM tones. Based on their response to tones of different duration, we classified the cells as short-pass, band-pass, long-pass, or untuned. Although the relative frequencies of these four groups were similar to results obtained in extracellular recordings in mice and rats (Brand et al. 2000; Pérez-
González et al. 2006; Xia et al. 2000), in our experiments both short-pass and band-pass experiments showed onset responses. It therefore seems inevitable that these cells would have been classified differently if we had used a long interval between tones. The cells we tentatively classified as short-pass most likely were cells with an onset response that recovered from synaptic adaptation more slowly than untuned cells with an onset response, rather than cells that were preferentially activated by brief tones. Similarly, the band-pass neurons most likely should be viewed as long-pass neurons that were more sensitive to the effects of adaptation. A special role for inhibition was not apparent in most band-pass neurons; the AHP sensitive to the effects of adaptation. A special role for inhibition was not apparent in most band-pass neurons; the AHP observed in some of the cells appeared to be attributable to the effects of the deactivation of $I_h$, as discussed in the following text. For the long-pass category, four of seven cells had a fast IPSP and these cells were characterized by an EPSP with a slow rise time. In these cells, inhibition therefore appears to be important in shaping the long-pass response (Klug et al. 2000; Pérez-González et al. 2006). In the other three cells, a role of inhibition was not apparent. We also did not observe evidence for a special role of inhibition in AM processing (Sinex et al. 2005; Walton et al. 2002), in agreement with the lack of effect of a pharmacological block of inhibition on phase locking (Burger and Pollak 1998; Caspary et al. 2002; Zhang and Kelly 2001).

The whole cell recordings allowed us to monitor the underlying membrane fluctuations that determine the firing responses to SAM tones of neurons in the IC. Our data are in agreement with the results of the two cells for which the responses to SAM tones were reported in Casseday and Covey (1992), who observed a cyclical input pattern, phase-locked to the envelope of the stimulus. Most cells showed a gradual decrease in the size of the evoked responses with increasing modulation frequency, which is in agreement with the observation that the temporal modulation transfer function is already low-pass at the level of the auditory nerve (reviewed by Joris et al. 2004). The observed cutoff frequencies were similar to phase-locked responses in extracellular recordings (e.g., Burger and Pollak 1998; Gooler and Feng 1992; Langner and Schreiner 1988; Rees and Möller 1983).

We compared the responses to tones of different duration and to SAM tones and found that the responses during the duration protocol, especially the persistency of the response to the last tone, were a good predictor for the size of the phase-locked response in the AM protocol. Cells that showed a relatively large, long-lasting response to the last tone in the duration protocol showed a relatively large response to the SAM tones as well. Therefore long-pass or band-pass cells did relatively well. In contrast, we observed that neurons that were classified as short-pass (i.e., cells with an onset response showing clear adaptation in the duration protocol) had a small phase-locked response to SAM tones. Cells with a low-plateau amplitude also responded poorly. We conclude that the ability to have a sustained response to tones is a key factor in determining the ability to respond to SAM tones with a phase-locked response. Our findings agree well with earlier studies, where it was also observed that onset neurons respond poorly to tones with relatively slow rise times, such as SAM tones (Condon et al. 1996; Gooler and Feng 1992; Sinex et al. 2002, 2005). Onset neurons will be excited more effectively by more rapidly changing tones, such as trains of brief tone bursts (Gooler and Feng 1992; Sinex et al. 2002).

Accommodation and response to tones

The relation between firing patterns during constant-current injection and the response to tones was less clear-cut than in the cochlear nucleus (Feng et al. 1994). We did find, however, that some general membrane properties of the cells—most notably the extent of accommodation during current injection, the presence or absence of bursts, and the presence or absence of $I_h$—were important in predicting the responses to tones. The major discrepancy between tone and current-injection responses was that about two thirds of IC neurons showed a sustained response to current injection, but only about one third responded in a sustained manner to tones, which is similar to what has been observed during extracellular recordings (Brand et al. 2000; Walton et al. 2002; Xia et al. 2000). Because the cells of the IC are at least one synapse further downstream, the input to the cells in the IC is subject to additional transformations in between the auditory nerve fibers and the IC, which are presumably responsible for the transient nature of the inputs. Nevertheless, we did observe that cells that fired in a sustained manner during tones, or whose EPSPs decreased only little during long tones, invariably showed little accommodation during current injection, indicating that little accommodation is a necessary, but not a sufficient, condition for a sustained response to long tones. Interestingly, none of the sustained or accommodating cells showed a good response to SAM tones. Although the sample was limited, we did observe that burst-sustained cells showed a good response to SAM tones, similar to stellate cells in the ventral cochlear nucleus, which also have an onset-chopper response to tones and a good phase-locked response to SAM tones (Frisina et al. 1990; Rhode and Greenberg 1994), whereas burst-onset cells did not show a good phase-locked response to SAM tones, suggesting that little accommodation is a necessary, but not a sufficient, condition for a good response to SAM tones.

We observed several other examples for a correlation between specific membrane properties and a role in sound transduction in the IC—foremost the presence or absence of $I_h$.

Cells with $I_h$ were more excitable

About half of the cells in the IC showed evidence for the presence of $I_h$. These cells showed a depolarizing sag in response to hyperpolarizing current injection and a hyperpolarizing sag during depolarizing current injection. We did not pharmacologically confirm that the sag was attributed to $I_h$, but this seems quite likely because frequency of occurrence and time course matched results in earlier slice studies in the rat, where pharmacology was more easily performed (Koch and Grothe 2003). Nevertheless, we cannot exclude a contribution of additional channels to the observed sag. $I_h$ appeared to play an important role in setting the excitability because cells with $I_h$ were more likely to respond with spikes to the tones. These cells had a more positive membrane potential and a somewhat lower action potential threshold than that of cells without $I_h$, whereas their EPSP sizes did not differ significantly, suggesting that the effect of $I_h$ on excitability was mostly by its effect on the membrane potential. Modulation of $I_h$ is therefore
Cells with $I_h$ were associated with pauser and chopper responses

Both chopper (Brand et al. 2000; Willott and Urban 1978; Xia et al. 2000) and pauser (Kuwada et al. 1997; Rees et al. 1997; Semple and Aitkin 1980) responses to tones were associated with the presence of $I_h$. These responses were characterized by an onset burst of activity in response to tones. The deactivation of $I_h$ seemed to be responsible for the cessation of activity after the onset response because a similar pattern was also observed during current injection, when synaptic inhibition does not need to be considered. The deactivation of $I_h$ may play a role as long as the spike thresholds are below the reversal potential for $I_h$, which has been reported to be between $-29$ and $-40$ mV in auditory neurons (Bal and Oertel 2000; Banks et al. 1993; Cuttle et al. 2001; Rodrigues and Oertel 2006), and as long as the resting membrane potential is sufficiently negative to allow activation of $I_h$. Based on the time course of the observed depolarizing or hyperpolarizing sag during current injection, these cells belong to the subset of IC neurons that show fast gating of $I_h$ (Koch and Grothe 2003). Intracellular recordings from onset-chopper D-stellate cells in the cochlear nucleus also show a clear hyperpolarizing sag during tones (e.g., Needham and Paolini 2006). Because these cells also have $I_h$ (Fujino and Oertel 2001; Rodrigues and Oertel 2006), a contribution of deactivation of $I_h$ may contribute to the onset-chopper response in these cells as well.

Because of the similarity of the time course of the hyperpolarizing sag during depolarizing current injection with the time course of the membrane potential in response to tones, we consider the deactivation of $I_h$ a more likely cause than synaptic inhibition for the cessation of firing in pauser responses (Banks and Sachs 1991; Needham and Paolini 2006; Rose et al. 1963). The pauser cells may differ from the other chopper cells by having a delayed excitatory input, as was observed in isolation in several long-pass neurons.

Cells with $I_h$ showed an AHP following long tones

In all cells with $I_h$ that showed a significant plateau depolarization during long tones, an AHP was observed. Because the kinetics matched the AHP observed in the same cells following depolarizing constant-current injection (Koch and Grothe 2003) we conclude that the reactivation of $I_h$ must have contributed to the AHP following long tones. Voltage-clamp experiments would be needed to assess a possible additional role of synaptic inhibition. We speculate that this AHP may contribute to off-suppression of spontaneous activity (Willott and Urban 1978) or, more generally, to forward masking. The responses in T-stellate cells of the cochlear nucleus, especially the C-T2-subtype (Paolini et al. 2005), show an adapting depolarization followed by an AHP after the end of a tone that was very similar to sustained responses in IC cells containing $I_h$, suggesting a similar function for $I_h$ in these cells.

In conclusion, despite the large variability in the responses to both tones and current injections between IC neurons, some clear patterns emerged. By defining a contribution of accommodation, burst firing, or the presence of a sag during constant-current injections to the response to tones, our data show for the first time how the different intrinsic firing patterns of IC neurons contribute to auditory processing. The wide availability of genetically modified mice may help to further dissect the relative contribution of membrane and synaptic properties in auditory transduction in the IC.

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**References**


