Intracellular Responses of Neurons in the Mouse Inferior Colliculus to Sinusoidal Amplitude-Modulated Tones

H.-R. Geis and J. G. G. Borst
Department of Neuroscience, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands

Submitted 26 August 2008; accepted in final form 30 January 2009

Geis H-R, Borst JGG. Intracellular responses of neurons in the mouse inferior colliculus to sinusoidal amplitude-modulated tones. J Neurophysiol 101: 2002–2016, 2009. First published February 4, 2009; doi:10.1152/jn.90966.2008. Changes in the temporal envelope are important defining features of natural acoustic signals. Many cells in the inferior colliculus (IC) respond preferentially to certain modulation frequencies, but how they accomplish this is not yet clear. We therefore made whole cell patch-clamp recordings in the IC of anesthetized mice while presenting sinusoidal amplitude-modulated (SAM) tones. The relation between the number of evoked spikes and modulation frequency was used to construct rate modulation transfer functions (rMTFs). We observed different types of rate tuning, including band-pass (16%), band-reject (13%), high-pass (6%), and low-pass (6%) tuning. In the high-pass rMTF neurons and some of the low-pass rMTF neurons, the tuning characteristics appeared to be already present in the inputs. In both band-pass and band-reject rMTF neurons, the nonlinear relation between membrane potential and spike probability ensured preferential spiking during only a small part of the modulation period. Band-pass rMTF neurons had rapidly rising excitatory postsynaptic potentials, allowing good phase-locking to brief tones and intermediate modulation frequencies. At low modulation frequencies, adaptation of their spike threshold contributed to the onset response. In contrast, band-reject rMTF neurons responded with small excitatory or inhibitory postsynaptic potentials to brief tones. In these cells, a power law could describe the supralinear relation between average membrane potential and spike rate. Differences in timing of synaptic input and presence or absence of spike adaptation therefore define band-pass and band-reject rate tuning to SAM tones in the mouse IC.

INTRODUCTION

Whereas cochlear nerve fibers may phase-lock to modulation frequencies as high as a few kilohertz, at the level of the auditory cortex the maximum frequencies have dropped to much lower values (Joris et al. 2004). This decrease in temporal fidelity at later stages of auditory processing necessitates a change in the way rapid temporal features are encoded, since signal periodicity is an important feature of auditory recognition and classification (Yost 1991). It has thus been argued that, gradually, a temporal code is traded in for a rate code (Joris et al. 2004). At the level of the auditory cortex, many neurons implicitly encode rapidly occurring events by increasing their firing rate. The inferior colliculus (IC) seems to play a transitional role between temporal representation of amplitude modulation (AM) in the periphery and a more rate-based code in the cortex (Langner and Schreiner 1988; Liang et al. 2002; Rose and Capranica 1985). Evidence has been presented that the IC contains a map for modulation frequencies that is orthogonal to the tonotopic organization, suggesting that extraction of modulation frequency by a rate code is a major task of the IC (Schreiner and Langner 1988).

The response of auditory neurons to amplitude modulations is generally tested using sinusoidal amplitude-modulated (SAM) tones at different modulation frequencies (Joris et al. 2004). Two types of response measures are commonly used to quantify responses of cells to SAM tones: one for spike rate and the other for synchronization of spikes to the modulation cycle. These measures are used to construct modulation transfer functions, relating spike rate to modulation frequencies (rate modulation transfer function [rMTF]) and the synchronization of spikes to the modulation cycle (temporal modulation transfer function [tMTF]). Almost all cells in the IC show either low-pass or band-pass tMTFs in response to SAM tones (Rees and Langner 2005). The rMTF of cells in the IC can be classified as band-pass, band-reject, low-pass, high-pass, complex, or all-pass (Rees and Langner 2005).

Which cellular mechanisms are underlying the different rate and temporal tuning classes in the IC? The few intracellular studies addressing this question have shown that the membrane potential of IC neurons can phase-lock to the modulation envelope in response to SAM tones (Casseday et al. 1994; Leary et al. 2008) and that the amplitude of this response decreases with higher modulation frequencies (Tan and Borst 2007). In addition, several models have been made for band-pass rate tuning to AM stimuli, but essential features of these models, such as coincidence detection (Borst et al. 2004; Guérin et al. 2006; Hewitt and Meddis 1994; Langner 1981), the presence of feedback (Friedel et al. 2007), or rate-tuned inhibition (Dicke et al. 2007; Nelson and Carney 2004, 2007), have not yet been tested.

Recent in vivo whole cell recordings from the IC have revealed an unexpected heterogeneity in the timing, amplitude, and frequency characteristics of both the inhibitory and excitatory inputs to IC neurons, with different neurons displaying onset, sustained, and/or offset inhibitory or excitatory postsynaptic potentials (IPSPs or EPSPs), depending on frequency and intensity (Tan and Borst 2007; Xie et al. 2007). We hypothesize that this heterogeneity contributes to differences between cells in both rate and temporal SAM tuning. Another mechanism that may contribute is the nonlinear relation between membrane potential and spike rate, which helps to define the difference between, for example, simple and complex cells in the visual cortex (reviewed in Priebe and Ferster 2008). To

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assess the contribution of input heterogeneity and the nonlinear relation between membrane potential and spike rate to both rate and temporal SAM tuning, we undertook a systematic study of the cellular mechanisms of SAM tuning by making in vivo whole cell patch-clamp recordings in the mouse IC. Band-pass and band-reject rate tuning classes differed considerably, both in the timing of their inputs and in the relative importance of synaptic inhibition. In addition, the nonlinear relation between membrane potential and spike probability contributed to sharpening of tuning in both classes. This relation was quite different in both classes, with adaptation of the spike threshold playing an important role in band-pass rMTF cells, but not in band-reject rMTF cells.

METHODS

Surgery

Animal procedures were approved by the Erasmus MC animal care ethics committee. Experiments were performed on a total of 48 C57Bl6 mice (postnatal day 21 [P21] to P35, male; Harlan). An intraperitoneal injection of ketamine/xylazine (65/10 mg/kg, respectively) introduced anesthesia. Anesthesia depth was assessed at least every 20 min by testing the hindpaw withdrawal reflex and checking for any signs of whisker movement; half of the initial dose of ketamine/xylazine was administered in case of a positive withdrawal reflex. Animals were placed on a homeothermic blanket with a rectal heating pole Bessel filter), and digitized at 25 kHz (Digidata 1322A) using Clampex. Sound intensities were calibrated between 0.5 and 65 kHz with a condenser microphone (ACO Pacific Type 7017, MA3 stereo microphone amplifier, TD SigCal) as described previously (Tan and Borst 2007). Experiments were conducted in a single-walled sound-attenuated chamber (attenuation ≥40 dB above 4 kHz; Gretch-Ken Industries, Lakeview, OR). Auditory stimuli were always presented contralaterally.

Tones of varying frequency–intensity combinations were presented to determine the frequency-response area. Frequencies ranged from 1 to 64 kHz in five steps per octave and intensities spanned 0 to 80 dB sound pressure level (SPL) in steps of 5 dB. Tones had a duration of 52 ms, including a 2-ms rise-decay time, and were repeated 10 times at an interval of 150 ms. The frequency that evoked either an IPSP (n = 21) or an EPSP (n = 46) at the lowest SPL was defined as the characteristic frequency (CF) and the corresponding SPL was defined as the minimal threshold (MT).

To study mechanisms underlying the tuning to SAM tones, in a first series of experiments the response to SAM tones was compared with the response to pure tones in a total of 30 cells. We will refer to the pure tones as unmodulated tones. Unmodulated tones were presented at CF and had durations of 2, 4, 8,..., 1,024 ms and a rise-decay time of 0.5 ms. For the SAM tones, CF was used as the carrier frequency; they had a modulation depth of 100%; modulation frequencies of 10, 20, 40,...,640 Hz; and a duration of 400 ms. The sine modulator had a phase shift of 90°, resulting in minimal amplitude (maximal down modulation) at the onset of the SAM tone. Both SAM tones and unmodulated tones were presented 30 dB above MT. Modulated and unmodulated tones were presented in fixed order, alternating between the two types of tones, with an interval ≥1 s between tones. This set of stimuli was repeated between 7 and 20 times, depending on the stability of the individual recordings.

In a second series of experiments, we systematically tested the influence of duration, rise time, and interval on SAM tuning in 37 cells. These unmodulated tones had durations of 1.56, 3.2, 6.24, 12.5, 25, 50, 100, and 400 ms, with the durations ≤100 ms matching the duration of a period of the SAM tones. Rise-decay times were 0.78, 3.2, and 50 ms, again chosen to be in the same range as the rise times of the different SAM tones. Intervals between these tones varied with the number of tone repetitions within the total 400-ms duration of a stimulus, ranging from 1, 2, 4, to ≤256 repetitions. The theoretical total number of stimuli was therefore 216 [i.e., (number of durations) × (number of rise-decay times) × (number of intervals)], but only stimuli that fit within the total duration of 400 ms were given, limiting the number to 69 different stimuli. These stimuli were compared with SAM tones with modulation frequencies ranging between 10 and 640 Hz. In addition, an isolated single cycle of modulation was presented for each frequency, yielding a total of 83 different stimuli. This set of stimuli was repeated 4–33 times in the same order, depending on the stability of the individual recordings. Intervals between stimuli were always ≥500 ms.

Histology

For histological confirmation of the recordings and the association between position and response properties of the cell, the recording...
electrode contained biocytin (0.5%). At the end of an experiment, animals were perfused transcardially (4% paraformaldehyde in 0.1 M phosphate buffer) and brains were processed as described by Horikawa and Armstrong (1988), with minor modifications. The likelihood of recovering the cell from which we recorded increased with recording time, which was, on average, 32 min (range 13–102 min) in the recovered cells. Dendritic labeling was very variable, with staining of the most proximal dendrites in 16 neurons, more extensive staining in 16 cells, and very extensive staining in 10 neurons. The three-dimensional (3D) position of neurons within the IC was determined by comparing coronal slices containing stained cells with slices from the reconstructed IC of a P25 C57Bl6 mouse.

Analysis of responses to SAM tones

Analysis was done in Clampfit 9.2 (Molecular Devices) or by using custom procedures written in the NeuroMatic environment (version 1.98, kindly provided by Dr. J. Rothman, University College London) within Igor Pro 6 (WaveMetrics, Lake Oswego, OR).

To identify rate-tuned neurons, the firing rates in response to SAM tones with modulation frequencies between 10 and 640 Hz were tested for homogeneity with an ANOVA. If the null hypothesis (i.e., the tones with modulation frequencies between 10 and 640 Hz were tested

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In a total of 15 neurons we injected sinusoidal currents, which evoked sinusoidal modulations of the membrane potential that resulted in phase-locked action potentials (Fig. 2A; Supplemental Figs. S2 and S3). At high injection frequencies, fluctuations of the membrane potential could be observed more easily by averaging across both sweeps and injection periods (Fig. 2B). The modulated potential (MP), the maximal peak-to-trough amplitude of these averaged fluctuations, decreased with increasing frequencies of the sinusoidal injection (Fig. 2, B, C, and D; Supplemental Figs. S2 and S3). Probably, filtering effects due to the passive membrane properties of the IC neurons were responsible for this low-pass behavior, although a quantitative assessment of their contribution was hampered by the high access resistance of our recordings. Interestingly, although the MP showed low-pass characteristics, this was not necessarily the case for the vector strength, a measure for phase-locking (Fig. 2, C and D; Supplemental Figs. S2B and S3B). Despite the decrease of the MP with increasing modulation frequency, the neuron showed a clear phase-locking response at 640 Hz (Fig. 2Bc), whereas this was not observed in the same cell in response to a 640-Hz SAM tone (Fig. 6, Ab and Bb). Six other cells also showed significant vector strength at an injection frequency of 640 Hz (Fig. 2D; Supplemental Figs. S2B and S3B), whereas these cells—as all other cells in this study—did not show a phase-locked response to 640-Hz SAM tones (Figs. 4D, 5E, and 6D).

In response to sinusoidal current injections, different types of rMTFs could be observed. Eight cells showed an all-pass rMTF (Supplemental Fig. S3), three cells complex tuning, three cells low-pass (Fig. 2), and one cell a band-pass rMTF (Supplemental Fig. S2). The type of tuning often differed from what was observed in response to SAM tones (cf. Figs. 2 and 6; Supplemental Fig. S2 and Fig. 4; Supplemental Fig. S3 and Fig. 5).

Our data show that constant-amplitude sinusoidal current injections of increasing modulation frequency, up to the highest tested frequency of 640 Hz, result in a decreasing MP, but that this decrease does not necessarily lead to a low-pass rMTF. In addition, these experiments show that IC neurons have the capability to phase-lock to frequencies >600 Hz in response to current injections.

**General response to SAM tones**

The response to SAM tones was tested in all neurons. The tones had a carrier frequency that was equal to CF and were modulated at frequencies of 10, 20, 40, . . . , 640 Hz (Figs. 3–7). In a first set of experiments, the response to SAM tones was compared with the response to unmodulated tones of different durations. At increasing modulation frequencies, both the rise time and the duration of the SAM tone envelope changes. In a second set of experiments, we therefore compared the response to SAM tones with the response to tones for which we systematically varied duration, interval, and rise-decay time, as detailed in METHODS. In most cells, the SAM tones of different frequencies led to a characteristic response pattern. At low frequencies, the synaptic inputs generally resulted in an MP identical to that of the modulation envelope. The average, truncated membrane potential is shown as a white overlay in Figs. 3–7A. If these fluctuations were large enough, they triggered phase-locked action potentials (Figs. 3–7Aa; raster plots are shown below membrane potentials). The MP decreased with increasing modulation frequencies (Figs. 3, D and E, 4D, 5E, 6, and 7, D and E, bottom), as was also observed following sinusoidal current injections (Fig. 2, C and D; Supplemental Figs. S2B and S3B). In contrast to sinusoidal current injections, the membrane potential did not show a phase-locked response at a modulation frequency of 640 Hz and the response to 640-Hz SAM tones generally was similar to the responses to long, unmodulated tones (cf. Figs. 3–7, Ac and Bc; Tan and Borst 2007). The response to intermediate modulation frequencies generally was more similar to the response to short tones (cf. Figs. 3–7, Ab and Bb). The onset of SAM tones at low modulation frequencies is slower; thus at low modulation frequencies, responses to SAM tones were better mimicked by tones with slower onset (Figs. 3–6Ba).

Analysis of the action potentials evoked by the SAM tones classically results in the construction of two types of modulation transfer functions (MTFs). A plot of the vector strength as a function of frequency results in the temporal MTF (tMTF; e.g., Fig. 3, D and E, middle). Only low-pass (\( n = 9 \); Fig. 4D) and band-pass (\( n = 30 \); Fig. 6D) tMTF were observed. A plot of the number of evoked action potentials as a function of frequency results in the rate MTF (rMTF; Fig. 3D, top). Based on statistical criteria, a total of 28 cells showed tuning of the firing rate to SAM tones. Of these rate-tuned cells, 11 were classified as band-pass, 9 as band-reject, 4 as low-pass, and 4 as high-pass. The remaining cells were untuned or showed complex tuning.

We will next describe the six different types of rMTF. We will first briefly summarize the heterogeneous group with complex or all-pass tuning. We then show examples of two
infrequently observed groups: the high-pass rMTF (Fig. 3) and low-pass rMTF (Figs. 4 and 5). Finally, the two main classes of rate tuning, cells with a band-reject or a band-pass rMTF, are described in detail (Figs. 6 and 7). A comparison of their properties illustrates the importance of both the timing of inputs (Figs. 8–10) and intrinsic spike-triggering mechanisms (Figs. 9 and 10) in defining SAM tuning in the IC.

All-pass and complex tuning

A total of 39 cells showed all-pass or complex rate tuning. Of these, 12 cells (18%) did not fire action potentials in response to tones of 30 dB above the minimal threshold. In response to long tones, 8 of these 12 neurons showed an EPSP, whereas in 4 cells, an IPSP could be observed. The absence of action potentials made determination of rate tuning impossible. Of these 12 cells, 10 cells did not fire action potentials to any of the simple tones (null-tuned neurons; Xie et al. 2007).

In the other 27 (40%) neurons with all-pass or complex rate tuning, action potentials were present. In 13 of these, the number of evoked action potentials was relatively low and no significant differences were detected with an ANOVA (Supplemental Fig. S4). In the remaining 14 neurons, the rMTF had a complex shape (Langner and Schreiner 1988). An example is shown in Supplemental Fig. S5.

High-pass tuning

A total of four cells (6%) showed a high-pass rMTF. Figure 3A shows a neuron that displayed an increase in firing rate with increasing modulation frequency. Responses to unmodulated tones are shown in Fig. 3B. In this neuron, action potentials were mainly triggered at the onset of the response. Stimuli with slow rise times (Fig. 3Ba), including SAM tones at low modulation frequencies (Fig. 3Aa), evoked few spikes. At increasing modulation frequencies, a larger onset depolarization resulted in more action potentials at the beginning of the response (Fig. 3, Ab and Bb). The response to a modulation frequency of 640 Hz and the response to a long, unmodulated tone were similar in shape (Fig. 3, Ac and Bc), but the 640-Hz SAM tone resulted in a slightly larger depolarization than the unmodulated tone (Fig. 3C) and triggered more spikes. Long,
unmodulated tones with rise times of 3.125 and 50 ms resulted in smaller depolarizations and triggered fewer spikes, indicating an effect of rise time on the onset response. The high-pass behavior was therefore due to the relatively strong depolarization at the onset of the response to the 640-Hz SAM tone, which was larger than the response following most of the other 82 stimuli to which this cell was subjected. The data for the different modulation frequencies are summarized in Fig. 3D.

Two cells displayed behavior similar to that of the cell illustrated in Fig. 3. Although they did not show an increase in depolarization and number of spikes with shorter rise time, they did show an adapting response to long, unmodulated tones. In all three neurons, brief tones resulted in onset EPSPs. The amplitude of these onset EPSPs was very variable between cells. The response to SAM tones with low modulation frequency was similar to the response to tones with slow rise and decay. The response to SAM tones at intermediate frequencies mimicked the response to a sequence of brief tones. At high modulation frequency, an increase in the size of the EPSP and the number of action potentials could be observed and this response was larger than the response to any other tone. At high modulation frequency, there was no evidence for a phase-

FIG. 2. Response to sinusoidal current injections. A: intracellular responses to 150-pA sinusoidal current injections at modulation frequencies of 10, 80, and 640 Hz are displayed in the top panels labeled a, b, and c, respectively. White overlay shows the average (12 repetitions) of truncated traces. Raster plots are displayed in the middle panel and stimulus is displayed in the bottom panel. Resting potential: −63 mV. B: membrane potential (top) generated by averaging the truncated membrane potentials over repetitions and periods and corresponding spike histogram (bottom, 30 bins per period), which was generated from the raster plots in A. Despite the small modulated potential (MP) at 640 Hz (top right), there is a clear change in spike frequency, which is in phase with the MP. The broken line indicates resting membrane potential. C: relation between firing rate during the current injection (top, averaged over stimulus repetitions), vector strength (middle, circles), or MP (bottom, crosses) and modulation frequency for the cell displayed in A. The rate tuning in response to sinusoidal amplitude-modulated (SAM) current injections was low-pass. Vector strength differed significantly from zero at all frequencies. D: relation between average firing rate (top), vector strength (middle), or MP (bottom) and modulation frequency for all cells with a response to sinusoidal current injections (n = 15 experiments). Before averaging, firing rates and MPs were divided by their respective maxima.
locked response. Although we cannot entirely exclude the presence of a phase-locked response in the dendrites, the lack of a somatic phase-locked response, in combination with the lack of evidence for an increase of synaptic inhibition at high modulation frequencies, suggest that in these three neurons the high-pass rMTF emerged more peripherally in the auditory pathway, in cells that can phase-lock to higher modulation frequencies.

The fourth high-pass neuron responded to unmodulated tones with nonadapting EPSPs, increasing in duration as stimulus duration increased. At low modulation frequencies, the response followed the modulation envelope of the SAM tone, evoking phase-locked action potentials. The cell showed little or no adaptation and the sustained firing at high modulation frequencies was responsible for the high-pass rMTF of this cell (results not shown).

A summary of the properties of these four cells is shown in Fig. 3E. Firing rate increased with modulation frequency, whereas the MP and the vector strength decreased. The vector strength was rarely significant, indicating poor phase-locking. We conclude that high-pass rate tuning in the majority of these cells most likely originated more peripherally in the auditory system.

**Low-pass tuning**

Low-pass rMTFs were observed in four cells (6%). Two distinct mechanisms appeared to underlie this behavior. Figure 4 illustrates one of the two neurons with a low-pass rMTF that showed an adapting response to unmodulated tones. At low modulation frequencies, most of the spikes were evoked at the beginning of every modulation period, with the EPSP amplitude already decreasing before the maximum intensity within each modulation cycle was reached (Fig. 4Aa). This cell showed relatively good phase-locking, with significant vector strength at a modulation frequency as high as 80 Hz (Fig. 4D, middle). At 80 Hz, however, the peak amplitudes of the EPSPs that were triggered by the individual modulation cycles did decrease during the tone, presumably due to adaptation (Fig. 4Ab). As a result, despite the larger number of modulation cycles, the total number of evoked spikes was lower at intermediate modulation frequencies than that at low modulation frequencies. At high modulation frequencies, spikes were triggered at the onset of the SAM tone, but the peak amplitude of the EPSP was lower than that at low modulation frequencies and the amplitudes again decreased during the tone (Fig. 4Ab). The responses to SAM tones could be mimicked by tones with similar rise-decay times and durations (Fig. 4B). A tone with a slow rise time evoked a response comparable to a single modulation period at low modulation frequency (Fig. 4, Aa and Ba). A brief tone with a rapid rise time resulted in a response that was similar to the response to SAM tones of intermediate frequency (Fig. 4, Ab and Bb). Similar results were obtained in the other neuron. Interestingly, both neurons showed a strongly accommodating response to square-pulse current injections (Fig. 4C), suggesting that voltage-dependent ion channels contributed to the adapting response during SAM tones.

The behavior of the other two neurons with a low-pass rMTF was very different from the cell shown in Fig. 4. These two neurons were both spontaneously active and their spontaneous activity was inhibited by SAM tones (Fig. 5A). The low-pass rMTF was due to an escape from the inhibition, which happened only at low modulation frequencies. Although the escape hap-
Band-pass tuning

Eleven cells (16%) showed a band-pass rMTF. Figure 6 shows a cell with an rBMF of 80 Hz. The cells with a band-pass rMTF responded to SAM tones at intermediate modulation frequencies with a series of onset EPSPs, which showed little adaptation during the tone. All band-pass neurons also responded with an onset EPSP to stimulation with short tones, which was similar to the response to one period of a SAM tone at intermediate modulation frequencies (cf. Fig. 6, Ab and Bb). These onset EPSPs were sufficiently large to trigger action potentials. A comparison of the MP and spike histogram showed that at a low modulation frequency, the cell illustrated in Fig. 6 still fired predominantly to the onset of the tone (Fig. 6Ca). With increasing modulation frequency the number of action potentials per modulation cycle decreased, until, between a modulation frequency of 40 and 80 Hz, it decreased slightly. Due to the increase in repetition rate, the total spike count still increased at these frequencies. The response to high-frequency SAM tones resembled the response to long, unmodulated tones; in both cases, most of the spikes were evoked at the onset of the tone. Figure 6D summarizes the responses of this cell. The EPSP evoked by long, unmodulated tones or 640-Hz SAM tones was adapting in eight neurons and the remaining three cells displayed an inhibited late response. As a result, fewer spikes were evoked at high modulation frequencies than at intermediate modulation frequencies. Since total spike number was also lower at lower modulation frequencies than that at intermediate frequencies, largely due to the lower number of modulation cycles, this led to a band-pass rMTF in these cells. Most of these neurons had an rBMF of 80 (n = 3) or 160 Hz (n = 5).

The tMTF could be determined in 10 of the neurons with a band-pass rMTF. The temporal best modulation frequency (tBMF) ranged from 20 to 320 Hz (median 60 Hz), giving all cells a band-pass tMTF. The band-pass cells, like the high-pass
cells of Fig. 3 and the low-pass cells of Fig. 4, showed a good response to brief tones and good phase-locking (Fig. 6E). In contrast to the low-pass cells, however, only one of the band-pass cells had an accommodating response to depolarizing current injections; most of them showed a sustained or burst-sustained firing pattern and one showed an accelerating firing pattern. A comparison of these three rate-tuning classes thus shows that the main difference between band-pass and low-pass cells was found in the strong accommodation of the low-pass cells, whereas the main difference between band-pass and high-pass cells was the small increase in depolarization at the highest modulation frequencies of the latter (Supplemental Fig. S6).

Band-reject tuning

Nine neurons (13%) displayed a band-reject rMTF. The rate worst modulation frequency (rWMF) was 80 Hz in eight cells and 40 Hz in the remaining neuron. The responses to SAM tones of a band-reject cell are displayed in Fig. 7A. This neuron showed a high firing rate in response to both 10- and 640-Hz SAM tones, whereas 80-Hz SAM tones did not evoke any spikes. A comparison of the response to unmodulated tones of different duration provides a simple explanation for the dramatic difference between the band-reject and the band-pass cells. Whereas the band-pass cells responded with an onset EPSP to a brief (<2 ms) tone, this band-reject cell showed an onset IPSP (Fig. 7Bb). This suggests that during stimulation at intermediate modulation frequencies, the repetitive action of the IPSPs prevented firing in this cell. In response to unmodulated tones of longer duration, the onset IPSP was followed by a delayed EPSP (Fig. 7, Ba and Bc). At low modulation frequencies, the SAM tone of a single modulation period lasted sufficiently long to evoke a delayed, suprathreshold EPSP (cf. Fig. 7Aa). At high modulation frequencies, the response became quite similar to the response to a long, unmodulated tone (cf. Fig. 7, Ac and Bc). A comparison of the MP and the modulation spike histogram shows the large modulation of the membrane potential at low modulation frequencies and the relatively hyperpolarized potentials reached at intermediate modulation frequencies (Fig. 7C). All neurons with a band-reject rMTF showed low tBMFs, ranging from 10 to 40 Hz (median 20 Hz). This resulted in two cells with a low-pass tMTF and seven neurons with a band-pass tMTF. The parameters of the cell shown in Fig. 7, A–C are summarized in Fig. 7D. The average over all band-reject neurons is displayed in Fig. 7E. For both the band-pass neurons and the band-reject neurons, there was not much variability in the rBMFs, as illustrated by the similarity between the shape of the example and the respective averaged rMTFs (cf. Fig. 6D vs. 6E or Fig. 7D vs. 7E).

Comparison between band-pass and band-reject rate tuning

Band-pass and band-reject were frequently observed rMTFs not only in this study but also in others (Condon et al. 1994, 1996; Langner and Schreiner 1988; Rees and Palmer 1989; Rose and Capranica 1985; Zhang and Kelly 2003). We therefore compared the response properties of neurons belonging to these two rate-tuning classes in more detail. From the results shown in Figs. 6B and 7B, it already becomes clear that these two classes differed in their response to tones of different

FIG. 6. Band-pass rate tuning to SAM tones resulting from onset EPSPs. A: the intracellular response to 10-, 80-, and 640-Hz SAM tones is shown in a, b, and c, respectively. The average of truncated potentials is shown as a white overlay. Raster plots are displayed in the middle panel and the stimulus is displayed in the bottom panel. CF: 12.1 kHz, SPL: 40 dB, resting potential: −58 mV, number of repetitions: 20. A and B: as in Fig. 3, A and B, respectively. C: MP (top) and spike histogram (bottom, 30 bins per period) generated from truncated averaged potentials and raster plots in A. The broken line indicates resting membrane potential. D: firing rate (top, averaged over stimulus repetitions), vector strength (middle, circles), and MP (bottom, crosses) plotted against modulation frequency for the cell displayed in A–C. Closed circles indicate vector strength significantly different from zero. Data shown in A–D are from the same cell as shown in Fig. 2, A–C. E: relation between average firing rate (top), vector strength (middle), or MP (bottom) and modulation frequency for all cells with a band-pass rMTF (n = 11 experiments). Before averaging, firing rates and MPs were divided by their respective maxima.
duration. This difference is summarized in Fig. 8 (top), which shows for both classes the average number of action potentials in response to unmodulated tones of different duration. Band-reject neurons responded rather poorly to unmodulated tones of short duration, but they fired many action potentials in response to long tones. With respect to duration tuning, these cells should therefore be classified as long-pass. In response to short tones, six cells showed an onset IPSP, whereas the remaining three neurons responded with a small, subthreshold onset EPSP. Long tones evoked adapting EPSPs in three cells and sustained EPSPs in the other six cells. An important defining feature of the band-reject neurons was thus the relatively large difference in the maximal depolarization reached in response to a brief or a long tone (Fig. 8, bottom). In contrast, in the band-pass neurons, the maximal depolarization was almost constant for the different durations. The total number of evoked action potentials increased only marginally with tone duration and most action potentials were evoked at the onset of the tone. With respect to duration tuning, these cells should therefore be classified as all-pass with a transient response (Pérez-González et al. 2006).

The difference in the temporal response of the two classes was also evident during presentation of SAM tones. As shown earlier, the band-pass rMTF cells had higher vector strength at intermediate modulation frequencies (Figs. 6E and 7E). In Fig. 9A, we display the response to 10-Hz SAM tones of the band-pass cell of Fig. 6 and the band-reject cell of Fig. 7 next to each other. Action potential firing mainly occurred at the rising phase of the averaged membrane potential in the band-pass neuron. In contrast, the band-reject cell showed the highest firing probability when the averaged membrane potential was most positive. In band-reject cells, the relation between the average membrane potential and spike rate was supralinear, for the period both preceding and following the maximal depolarization; in both cases, this relation could be described by a power law (Fig. 9B, right). This relation could be approximated by a power-law function with a similar exponent at a modulation frequency of 20 Hz (Supplemental Fig. S7), indicating that the average membrane potential provides a good estimate for the instantaneous spike probability in the band-reject cell. The supralinear relation between average membrane potential and spike rate indicates that the tuning for spikes is much sharper than that for changes in the average membrane potential for this band-reject neuron. Similar results were obtained for all band-reject rMTF neurons.

In the case of the band-pass cell, the relation between average membrane potential and spike rate could be described by a power law only for potentials following the maximal depolarization (Fig. 9B, left, closed circles; Supplemental Fig. S7). The relation between firing rate and potentials preceding the maximal depolarization was not supralinear, and fits yielded values for $m < 1$ (Fig. 9B, left, open circles). The highest firing rates resulted from depolarizations just above the firing threshold and, with further depolarization, the spike rate went down, instead of up. Similar results were obtained for all band-pass rMTF neurons. In conclusion, both band-pass and band-reject neurons showed a nonlinear relation between the average membrane potential and probability of spiking. As a result, for both tuning classes, tuning for synaptic potentials was broader than that for spikes. For band-reject neurons, the spike probability could generally be well predicted from the average membrane potential, whereas this was not the case for the band-pass neurons, which showed time-dependent decreases in spike probability.
On average, spikes occurring late during the modulation period were triggered at more positive potentials than early spikes, suggesting an adaptation of the firing threshold. Figure 9C shows the averaged, aligned action potential occurring either early or late during the 10-Hz modulation period. On average, late spikes were triggered at a more positive potential in the band-pass neuron (Fig. 9C, left), indicating an adaptation of the firing threshold. The difference was much smaller for early and late action potentials in the band-reject neuron (Fig. 9C, right). To investigate why excitability on average decreased in the band-pass, but not in the band-reject rMTF neurons, we measured spike threshold as a function of modulation period (Fig. 9D). On average, spikes occurring late during the modulation period were triggered at more positive potentials than early spikes in band-pass, but not in band-reject neurons. The relation between rate of rise of the EPSP preceding a spike and modulation period is displayed in Fig. 9E, showing a relatively uniform distribution for both the band-pass neuron, whereas in the band-reject neuron a negative correlation was observed ($r = -0.36; P < 0.001$). At 10 Hz, a significant, negative correlation was observed in a total of 4 of the 9 band-reject neurons and 1 of 10 band-pass cells.

The difference in firing threshold between early and late spikes is summarized in Fig. 10A for both band-pass and band-reject neurons, illustrating the adaptation of the spike threshold in band-pass but not in band-reject rMTFs. The adaptation of the spike threshold helps to explain why there are relatively few late spikes in band-pass neurons, but it does not explain why band-pass neurons—in contrast to band-reject neurons—respond well to brief tones. A comparison of the rate of rise of the EPSP preceding a spike, averaged over all modulation frequencies, showed a significant difference between all cells (ANOVA, $F = 2.765; P < 0.05$). Band-pass neurons showed a significantly larger rate of rise than that of band-reject neurons (Fig. 10B: $4.47 \pm 0.55$ vs. $2.25 \pm 0.23$ mV/ms; Tukey’s HSD, $P < 0.05$).

In conclusion, in both the band-pass and the band-reject rMTF neurons, most spikes occur during only a small part of the modulation cycle. In the case of the band-pass cells, the relatively rapidly rising EPSPs allow spikes to occur early. Due to adaptation of the spike threshold, a larger depolarization is needed at later time points. In the case of the band-reject neurons, the EPSPs triggering a spike rise more slowly and spikes occur later. There is only little evidence for adaptation, but due to the power-law relation between average membrane potential and spike rate, most spikes occur only during a restricted part of the modulation cycle, as for the band-pass neurons.

**General comparison of rate-tuning classes**

Responses of the cells in the different rate-tuning classes indicated that synaptic inputs were quite important in defining rate-tuning class membership. Mean age of the animal, characteristic frequency, minimal threshold, bandwidth at 80 dB, minimum latency to either IPSP or EPSP, membrane resistance, membrane time constant, relative size of depolarizing sag, and time constant of $I_h$ activation did not differ significantly between cells with high-pass, low-pass, band-reject, or band-pass rate tuning to SAM tones (ANOVA, $P > 0.05$). The only parameter showing a significant difference between the four rMTFs was the resting membrane potential during the experiment ($F = 3.372, P < 0.05$). The membrane potential during the experiment was significantly more positive in band-reject cells compared with high-pass neurons (Tukey’s HSD, $P < 0.05$), in agreement with the important role of synaptic inhibition in many of the neurons in the band-reject group.

To test for a spatial organization of the tuning classes or any of the other parameters mentioned in the previous paragraph, we color-coded the retrieved neurons within the 3D reconstruction for these different properties. In addition to the dorsolateral to ventromedial gradient of the characteristic frequency (Fig. 1), we also observed a dorsolateral to ventromedial gradient of increasing membrane time constant ($r = 0.392, P < 0.05$; Supplemental Fig. S8). For the other properties, no obvious dependence was observed.

**Discussion**

Using in vivo whole cell patch-clamp recordings, we describe cellular mechanisms for both rate and temporal tuning to sinusoidal amplitude-modulated tones in the mouse inferior colliculus. We observed that differences in synaptic inputs and the way spikes were triggered made important contributions to rate tuning in band-reject and band-pass cells, the two most prevalent rate-tuning classes.

**Relative frequency of rate-tuning types**

In our study, only 42% of neurons showed rate tuning to SAM tones, much less than that in earlier extracellular studies.
A major reason for this discrepancy comes from our definition of the characteristic frequency (CF). We defined CF as the lowest frequency that elicited either an EPSP or an IPSP and tested the SAM tones at 30 dB above the minimum threshold (MT). In about 40% of the neurons, there were few or no spikes elicited at that intensity, whereas in extracellular studies, CF is always defined on the basis of spikes. Other differences are the use of a statistical method (ANOVA) to define tuning, whereas with the 50% change criterion used in other studies (Langner and Schreiner 1988; Rose and Capranica 1985), the tuning criterion is more easily met at low firing rates. In addition, there are differences in the SAM stimuli that were used, with studies differing in the use of tonal carriers versus a noise carrier, depth of modulation, range of modulation frequencies, or SPL. Nevertheless, the relative proportion of rMTFs was in general agreement with earlier, extracellular studies. MTFs based on spike rate most often were band-pass, as also observed in guinea pig, cat, bat, frog, rat, and gerbil (Condon et al. 1994, 1996; Heil et al. 1995; Langner and Schreiner 1988; Rees and Palmer 1989; Rose and Capranica 1985; Zhang and Kelly 2003). Similar to our findings, band-reject MTFs were also observed relatively frequently in the rat and gerbil (Krebs et al. 2008; Zhang and Kelly 2003), whereas in other species, this tuning type was less common. The percentage of neurons displaying a low-pass or high-pass rMTF in rat was similar to what we observed (Zhang and Kelly 2003). In contrast, high-pass rMTFs were more common in bat (Condon et al. 1996) and low-pass rMTFs were more often observed in frog and cat (Langner and Schreiner 1988; Rose and Capranica 1985).
Intracellular recordings peripherally from the IC may shed postsynaptic potentials in response to SAM tones at the rBMF. In the high-pass rMTF cells, the tuning appeared to result from a nonmonotonic relation between spike rate and membrane potential. In the other low-pass rMTF cells, with their supralinear power-law relation, had low-pass behavior and the largest MP was always measured at modulation frequencies below the rBMF in band-pass rMTF cells (Fig. 6). The modulated potentials at SAM modulation frequencies below the rBMF were observed, the list of possible mechanisms is likely not exhaustive.

Cells with a band-reject or a band-pass rMTF were observed relatively often. They showed great differences in the timing of excitatory inputs. Band-pass rMTF neurons displayed good phase-locking properties and an onset response to tones, in agreement with earlier findings (Gooler and Feng 1992; Langner et al. 1987; Sinex et al. 2002), whereas band-reject neurons showed delayed excitatory inputs and often onset inhibition. Earlier studies have shown that onset neurons respond poorly to tones with relatively slow rise times, such as SAM tones (Condor et al. 1996; Gooler and Feng 1992; Sinex et al. 2002, 2005). Although inhibition plays an essential role in some models for band-pass rate tuning (Dike et al. 2007; Nelson and Carney 2004, 2007), we did not observe experimental evidence for this, in agreement with the observed lack of effect of blocking inhibition on the band-pass rMTF (Burger and Pollak 1998).

Role of coincidence detection in band-pass rate tuning

In many of the models of band-pass rate tuning, the IC neuron functions as a coincidence detector. The coincident input can be brought about by feedback or by feedforward delay lines (Friedel et al. 2007), by the convergence of many neurons with identical, or similar, narrowly tuned rMTFs (Guérin et al. 2006; Hewitt and Meddis 1994) or by the convergence of the output of different components of a network model (Borst et al. 2004; Langner 1981). The key feature of these models is that around the rBMF, the inputs are more synchronized, leading to an increased spike rate. Presumably, to be effective, the more synchronized input at rBMF should lead to an increase in the MP, whereas we typically observed low-pass behavior and the largest MP was always measured at modulation frequencies below the rBMF in band-pass rMTF cells (Fig. 6). The modulated potentials at SAM modulation frequencies corresponding to the rBMFs of VCN neurons (generally >150 Hz; Frisina et al. 1990; Rhode and Greenberg 1994) were quite small, indicating that other mechanisms must play an important role, as also suggested by the results of the sinusoidal current injections.

Sinusoidal current injections show how a low-pass MP can lead to a band-pass rMTF

Almost invariably, the MP decreased with increasing modulation frequency of the SAM tones. This was also observed during sinusoidal current injections, suggesting that the resistive–capacitive properties of the IC neurons contributed to this decrease. How can a low-pass MP result in a band-pass rMTF? Interestingly, during sinusoidal current injections, the firing rate could also increase at a higher modulation frequency, despite the decrease in the MP, leading to complex tuning or a band-pass rMTF. One reason for this was the contribution of the spike threshold. At low modulation frequencies, it was possible that the membrane potential was above the spike threshold level for a smaller proportion of the time than at intermediate modulation frequencies, where, due to filtering

Cellular mechanisms underlying rate tuning

Neurons with a low-pass or a high-pass rMTF were observed relatively infrequently. In two neurons, the low-pass rMTF most likely resulted from a nonmonotonic relation between sound-induced responses and sound level. In the other low-pass or in the high-pass rMTF cells, the tuning appeared to already present in the inputs, since these cells showed larger postsynaptic potentials in response to SAM tones at the rBMF. Intracellular recordings peripherally from the IC may shed more light on the cellular mechanisms underlying low-pass or high-pass rate tuning. In addition, because of the low number of cells with a low-pass or a high-pass rMTF from which we recorded, the list of possible mechanisms is likely not exhaustive.

Both during sinusoidal current injections and in response to tones, we observed that small changes in the average membrane potential could give rise to large changes in spike rate. The maximal vector strength in the IC is typically higher than that in the cochlear nucleus (reviewed by Joris et al. 2004). By decreasing the width of the cycle-averaged spike histograms, the observed supralinear relation between spike rate and average membrane potential will contribute to this sharpening of tMTFs.

In the band-reject rMTF cells, the relation between average membrane potential and spike rate could be well described by a power law. A consequence of the power-law relation is that the relative increase in the firing rate at the peak compared with the trough of the cycle-averaged membrane potential will be independent of the absolute firing rate at the trough, as long as this rate is greater than zero (Hansel and van Vreeswijk 2002; Miller and Troyer 2002). At higher modulation frequencies, modulations of the membrane potential were generally riding on top of a steady depolarization. However, since the relation between the MP and modulation frequency was almost invariably low-pass, the power-law relation implies that even if modulations occur on top of a steady-state component, the resulting tMTF will still be low-pass. Indeed, band-reject rMTF cells, with their supralinear power-law relation, had low tBMFs.

In contrast, band-pass rMTF cells, where the relation between average membrane potential and spike rate often showed time-dependent changes due to adaptation of spike threshold, often had a tBMF >40 Hz. This finding is in agreement with extracellular recordings showing that onset units exhibited band-pass tMTFs (Krishna and Semple 2000; Nelson and Carney 2007). At low modulation frequencies, firing was generally at the onset and, at later times, spike threshold adaptation reduced firing rates, yielding a skewed spike histogram and low vector strength. Spike threshold adaptation has also been observed during in vivo recordings in the hippocampus (Henze and Buzsáki 2001) and may contribute to tuning in both the somatosensory (Wilent and Contreras 2005) and visual cortex (Azouz and Gray 2003), illustrating that these mechanisms are not unique for the band-pass rMTF neurons of the IC.

Supralinear relation between membrane potential and firing properties contributed to temporal tuning

Role of coincidence detection in band-pass rate tuning

Sinusoidal current injections show how a low-pass MP can lead to a band-pass rMTF

What is the relation between average membrane potential and spike rate in band-pass rMTF cells? The relation between average membrane potential and spike rate could be well described by a power law. A consequence of the power-law relation is that the relative increase in the firing rate at the peak compared with the trough of the cycle-averaged membrane potential will be independent of the absolute firing rate at the trough, as long as this rate is greater than zero. This is in agreement with the extracellular recordings showing that onset units exhibited band-pass tMTFs. At low modulation frequencies, firing was generally at the onset and, at later times, spike threshold adaptation reduced firing rates, yielding a skewed spike histogram and low vector strength. Spike threshold adaptation has also been observed during in vivo recordings in the hippocampus and may contribute to tuning in both the somatosensory and visual cortex. These mechanisms are not unique for the band-pass rMTF neurons of the IC.

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effects, the MP could be riding on a steady depolarization. At high modulation frequencies, adaptation could contribute to a decrease in the firing rate. As a result, a band-pass rMTF could be obtained in many cells in response to SAM tones, and in one cell in response to sinusoidal current injections, even if the MP showed low-pass characteristics.

Role of inhibition in band-reject rate tuning

In the gerbil IC, rMTFs with a suppressive region were observed in neurons with sustained or pauser peristimulus time histograms (Krishna and Semple 2000). It was speculated that inhibitory inputs are responsible for these suppressive regions. However, bicuculline did not affect the shape of the rMTF in band-reject neurons in rats (Zhang and Kelly 2003). Our data partly support the hypothesis that inhibition is involved in creating the band-reject rMTF, since many of the band-reject neurons showed onset IPSPs. The band-reject neuron illustrated in Fig. 7 clearly showed a contribution of onset IPSPs to rate tuning: at intermediate modulation frequencies, the membrane potential remained below the threshold (Peña and Konishi 2002; Priebe and Ferster 2008). However, in other band-reject rMTF cells, we did not observe evidence for the presence of onset inhibition, indicating that it is not an absolute requirement. In these cells, a sluggish excitatory response appeared to be sufficient for the lack of response at intermediate modulation frequencies. In the frog auditory midbrain, long-pass duration-tuned cells also show a band-reject rMTF in response to SAM tones. This suggests that onset inhibition and sluggish onset excitation are two mechanisms creating band-reject rMTF that are conserved across evolution or, alternatively, are due to parallel evolution (Edwards and Rose 2003; Leary et al. 2008).

Was the timing of excitatory inputs sufficient to determine rate tuning or were the cellular properties of neurons with a band-pass and band-reject rMTFs also different? Apart from a difference in membrane potential, no obvious differences could be found. A comparison of spike-triggering mechanisms identified the rate of rise of the EPSP preceding the spikes as an important distinguishing feature between band-pass and band-reject neurons. We therefore conclude that differences in the timing of inputs and the resulting rate of change of the membrane potential play an important role in setting up the two main classes of rate tuning to SAM tones in the mouse IC, but that cellular mechanisms such as spike adaptation also contributed.

Significance of SAM tuning

SAM rate tuning often was not present in the inputs. For example, in the band-reject rMTF neurons, in many cases the interaction of inhibitory and excitatory inputs contributed to tuning. This suggests that in these cases, this type of tuning emerged within the cells from which we recorded. Therefore this is an example of a direct neuronal computational mechanism for a complex auditory feature. However, this observation does not necessarily mean that decoding SAM tones is a major task of these neurons. In the cells in which we tested a large number of stimuli, we found that responses to other types of tones were generally able to drive these neurons better than the SAM tones. This is in agreement with other studies demonstrating the dependence of AM tuning on parameters such as SPL, modulation depth, rise times, and duty cycle (Krebs et al. 2008; Krishna and Semple 2000; Rees and Möller 1987; Sinex et al. 2002). We also did not find evidence for a spatial organization of SAM tuning (Schreiner and Langner 1988), although we cannot exclude the possibility that with a larger sample, an organizational pattern would have emerged. Nevertheless, we expect that the cellular mechanisms that determine rate and temporal tuning to SAM tones are also important for more complex, natural sounds, including vocalizations.

A general finding within the IC is the enormous heterogeneity of the responses to tones (reviewed by Ehret and Schreiner 2005). For example, there is a large variability in the timing and duration of inhibitory and excitatory inputs (Casseday et al. 1994; Kuwada et al. 1997; Tan and Borst 2007; Xie et al. 2007, 2008). Our results show how the timing of excitatory and/or inhibitory inputs can be used to generate certain modulation transfer functions. Considering the complexity and heterogeneity of sound responses of individual cells within the IC, the tuning mechanisms for the band-pass and band-reject classes were remarkably simple.

The comparison of the band-reject and the band-pass rMTF neurons shows that they must serve quite different functions. The band-pass neurons will be able to respond to rapid fluctuations in the sound. Not only because of their onset response, but also due to the rapid adaptation of their spike threshold to ongoing tones, they will be specialized in detecting transient stimuli. In contrast, the band-reject neurons will not respond to brief tones but will continue to respond to ongoing tones since they show little adaptation of their spike threshold. The non-linear relation between spike rate and average membrane potential is expected to enable them to accurately represent slow modulations in amplitude. These two types of neurons are therefore important building blocks for the representation of complex sounds by the IC.

ACKNOWLEDGMENTS

We thank M. van der Heijden, S.W.F. Meenderink, and J.A.M. Lorteije for commenting on an earlier version of this manuscript and M. L. Tan for advice on in vivo patch clamping.

GRANTS

This work was supported by Neuro-Bisik Grant BSIK 03053 (SenterNovem, The Netherlands) and the Heinsius-Houbolt Fund.

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