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The Representation of Amplitude Modulations in the Mammalian Auditory Midbrain

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Krebs B, Lesica NA, Grothe B. The representation of amplitude modulations in the mammalian auditory midbrain. *J Neurophysiol* 100: 1602–1609, 2008. First published July 9, 2008; doi:10.1152/jn.90374.2008. Temporal modulations in stimulus amplitude are essential for recognizing and categorizing behaviorally relevant acoustic signals such as speech. Despite this behavioral importance, it remains unclear how amplitude modulations (AMs) are represented in the responses of neurons at higher levels of the auditory system. Studies using stimuli with sinusoidal amplitude modulations (SAMs) have shown that the responses of many neurons are strongly tuned to modulation frequency, leading to the hypothesis that AMs are represented by their periodicity in the auditory midbrain. However, AMs in general are defined not only by their modulation frequency, but also by a number of other parameters (duration, duty cycle, etc.), which covary with modulation frequency in SAM stimuli. Thus the relationship between modulation frequency and neural responses as characterized with SAM stimuli alone is ambiguous. In this study, we characterize the representation of AMs in the gerbil inferior colliculus by analyzing neural responses to a series of pulse trains in which duration and interpulse interval are systematically varied to quantify the importance of duration, interpulse interval, duty cycle, and modulation frequency independently. We find that, although modulation frequency is indeed an important parameter for some neurons, the responses of many neurons are also strongly influenced by other AM parameters, typically duration and duty cycle. These results suggest that AMs are represented in the auditory midbrain not only by their periodicity, but by a complex combination of several important parameters.

INTRODUCTION

Natural sounds such as human speech contain prominent amplitude modulations (AMs), and numerous psychophysical studies have shown that AMs are an important cue for a variety of tasks including speech recognition (Shannon et al. 1995), pitch perception (Rossing and Houtsma 1986), and stream segregation (Grimault et al. 2002). The behavioral importance of AMs has motivated the use of AM stimuli in many physiological studies of temporal processing in auditory neurons (for reviews, see Frisina 2001; Joris et al. 2004; Langner 1992). However, despite a wealth of experimental data, the representation of AMs in the responses of neurons at higher levels of the auditory pathway remains a topic of debate. The complexity of AMs as an acoustical property may play an important role in this issue.

The majority of physiological studies involving AM stimuli have used sinusoidally amplitude modulated (SAM) stimuli in which the envelope of a high-frequency "carrier" tone is specified by a low-frequency "modulator" tone. These studies have shown that the responses of many neurons in the ascending auditory pathway are strongly dependent on the frequency of the modulator. For example, many neurons in the central nucleus of the inferior colliculus (ICC) display strong tuning to modulation frequency with a diversity of tuning functions (high-pass, band-reject, etc.), leading to the hypothesis that AMs are encoded by their periodicity in the auditory midbrain (Krishna and Semple 2000; Langner and Schreiner 1988).

The use of the fact that neuronal responses are dependent on the modulation frequency of SAM stimuli as evidence that AMs in general are represented by their periodicity in the auditory midbrain is problematic for two reasons. The first reason is that tuning to the modulation frequency of SAM stimuli in the midbrain is not invariant to changes in stimulus context. For example, the tuning functions of neurons in the ICC can change dramatically with changes in the mean level, modulation depth, or background noise level of the stimulus, indicating that AMs with the same periodicity can have different neural representations (Krishna and Semple 2000; Rees and Palmer 1989). The second reason, which is the motivation for this study, is that AMs are a complex acoustical property, defined not only by modulation frequency, but also by a number of other important (and related) parameters. As shown in Fig. 1, changing the modulation frequency (F_{MOD}) in SAM stimuli causes systematic changes in other parameters: the duration of the positive phase of each cycle (DUR), and the pause interval between cycles (IPI). Only the duty cycle [DC = DUR/(DUR + IPI)] remains constant. Thus the relationship between neural responses and modulation frequencyfor AMs in general cannot be unambiguously defined using SAM stimuli alone, because apparent tuning to modulation frequency may in fact result from the covariation between modulation frequency and another parameter. The effects of this ambiguity have already been shown in the auditory brain stem, where comparison of responses to SAM stimuli and pulse trains suggests that, for many neurons, apparent tuning to modulation frequency in SAM stimuli is actually a reflection of tuning to changes in IPI (Grothe et al. 2001).

In this study, we investigate the representation of AMs in the auditory midbrain by analyzing responses to a series of pulse trains in which DUR and IPI are varied systematically, allowing us to quantify the importance of DUR, IPI, DC, and F_{MOD} independently. Although F_{MOD} is indeed an important parameter for some neurons, we find that the responses of many

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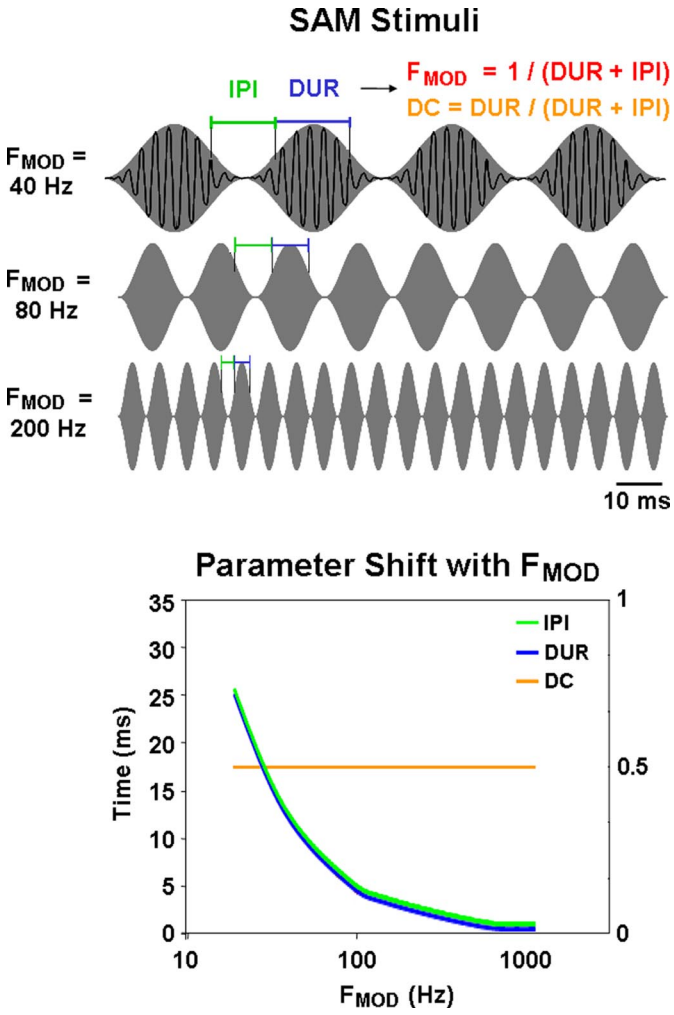

FIG. 1. The effect of changes in modulation frequency on other parameters of sinusoidal amplitude modulation (SAM) stimuli. The schematic diagrams show the interpulse interval (IPI) and duration (DUR) for SAM stimuli with modulation frequency F_{MOD} = 40, 80, and 200 Hz. The *bottom plot* shows the interdependence of IPI, DUR, and duty cycle (DC) on F_{MOD} for SAM stimuli. The *left vertical axis* applies to IPI and DUR. The *right vertical axis* applies to DC.

neurons are also strongly sensitive to changes in other parameters, typically DUR or DC. These results suggest that AMs are represented in the auditory midbrain not simply by their periodicity but by complex combinations of several important parameters. Parts of this study have been published in abstract form (Breutel et al. 2001).

METHODS

Physiological recordings

We recorded action potentials from single neurons in the ICC of 26 Mongolian gerbils (*Meriones unguiculatus*). The surgical procedures used in this study have been described in detail previously (Siveke et al. 2006). All experiments were approved according to the German Tierschutzgesetz (Reg. Obb. 211-2531-40/01). Briefly, adult gerbils were anesthetized by an initial intraperitoneal injection (0.5 ml/100 g body weight) of a physiological NaCl solution containing ketamine (20%) and xylazine (2%). During surgery and recordings, a dose of 0.03 ml of the same mixture was applied subcutaneously every 20 min. A small metal rod was mounted on the frontal part of the skull and used to secure the head of the animal in a stereotactic device during recordings. The animal was positioned in a sound-attenuated chamber, and a craniotomy was made over the inferior colliculus, 1.3–2.6 mm lateral from the midline and 0.5–0.8 mm caudal from the bregma. The dura mater overlying the cortex was removed, and glass pipettes filled with 2 M sodium acetate (impedance 10–30 MΩ) or tungsten electrodes (impedance 5 MΩ) were advanced into the inferior colliculus (2–4 mm below the surface). Recording of action potentials and stimulus generation was controlled by custom-made software (B. Warren, University of Washington, Seattle, WA). Recorded signals were amplified, filtered, discriminated, and fed into a computer via a DSP board (System II, Tucker Davis Technologies). Pressure-injected fluorescent latex beads (Lumafluor) or iontophoretically applied horseradish peroxidase (HRP, 720 nA for 8 min, Sigma) were used to verify that recording sites were in the central nucleus of the inferior colliculus.

Acoustic stimulation

Acoustic stimuli were delivered using Tucker Davis Technologies System II, controlled by custom made software, presented via electrostatic speaker driver ED1 (TDT System 3), and calibrated electrostatic speaker (TDT System 3) connected via a tube to the ear contralateral to the recording site. After electrophysiological isolation of a single neuron, pure tone stimuli of 40-ms duration (rise-fall time adjusted to avoid any spectral artifacts) were presented to the contralateral ear at various frequencies and levels. From responses to these stimuli, best frequency (BF), threshold, and response type (based on the classification scheme outlined in Rees et al. 1997) were determined. Next, SAM tones (carrier at BF, 20 dB above threshold, 100% modulation depth) and noise (20 dB above threshold, 100% modulation depth) were presented. For SAM noise stimuli, the bandwidth of the noise was centered on the BF and was adjusted to evoke to maximum spike rate (but was ≥1,000 Hz). SAM stimuli were of 150-, 200-, or 250-ms duration. F_{MOD} ranged from 20 to 1,000 Hz, presented in 11 logarithmic steps.

For some neurons, trains of five or more trapezoidal pulses (linear rise/fall, carrier at BF, 20 dB above threshold, 100% modulation depth) were also presented. The DUR of the pulses and the IPI were systematically varied, which resulted in corresponding changes in DC and F_{MOD}. The values of DUR (time during which the pulse was >50% of its maximum value) and IPI (time during which the pulse was <50% of its maximum value) used for each neuron were adjusted to an appropriate range based on observed response properties. For all neurons, pulse trains with at least five different values of DUR and IPI were presented. Of the 37 cells from which we recorded responses to both SAM tones and pulse trains, 24 had SAM rate modulation tuning functions (rMTFs), for which measurement of a preferred F_{MOD} was appropriate (i.e., a low-pass, band-pass, or high-pass rMTF; for all-pass and band-reject rMTFs, measurement of the preferred modulation frequency is less meaningful). For 19 of these 24 cells, the range of values for IPI and DUR in the pulse trains was such that the F_{MOD} of one or more of the pulse trains was similar to the preferred F_{MOD} for SAM tones (although not necessarily with a 50% DC). The rise/fall time of the pulses was 1 ms. It should be noted that the linear rise/fall of the pulses introduces additional frequencies into the stimulus that are not present in, for example, SAM stimuli. However, of the 37 cells from which we recorded responses to pulse trains, only 5 had a BF between 0 and 3.5 KHz, the range of frequencies for which the bandwidth of the pulse train stimuli exceeds the critical bandwidth of the auditory filter (Schmiedt 1989). Stimuli were presented in a randomized order (interleaved) with ≥10 repetitions of each stimulus. Stimulus repetition rates were chosen to avoid hysteresis effects (in most cases between 500 and 1,000 ms).

Data analysis

For all periodic stimuli, only the response to the ongoing component was analyzed. The response to the first pulse or first cycle of SAM stimuli and at least the first 10 ms of the response were omitted. For neurons with OFF discharges, the response correlated with the stimulus end was omitted from the analysis. For each neuron, the shape of the rMTF (the tuning function relating the spike rate of the ongoing response component to the F_{MOD}) was classified based on the range of frequencies for which the response was >50% of the maximum. To assess the effects of DUR, IPI, DC, and F_{MOD} on neural responses, partial Kendall rank correlation coefficients were computed. The partial correlation coefficient (CC_p) quantifies the effects of each stimulus parameter on the neural response independently, removing any ambiguity that arises because of covariations between different stimulus parameters.

To identify groups of neurons with similar response properties, each neuron was defined in a four-dimensional space by its CC_p for DUR, IPI, DC, and F_{MOD}. Neurons were sorted into clusters based on Euclidean distance. We used the median linkage algorithm, which creates clusters based on a weighted center of mass distance (WPGMC). This algorithm yielded robust results in which the mean distance from each neuron to the centroid of its assigned cluster was much smaller than the mean distance between the centroids of different clusters.

RESULTS

We recorded from 115 well-isolated single neurons within the ICC. The BFs of the neurons ranged from 0.8 to 45 KHz (only 6.9% with BFs <2 KHz). Response types for pure tone stimuli included sustained discharges (18%, 20/115; including build-up and pauser responses), transient ON or OFF responses (44%, 50/115), primary-like responses (32%, 38/115; strong onset with a weak ongoing component), and chopper responses (6%, 7/115). None of the cells displayed any phase-locking to pure tones. The spontaneous spike rates of the cells ranged from 0.1 to 139 Hz. On average, the response properties of the cells recorded with glass pipettes and tungsten electrodes were not significantly different.

Responses to SAM stimuli

In 95 of the 115 cells, we observed a response to SAM tone stimuli (pure tone carrier at BF, 20 dB above threshold, 100% modulation depth) that consisted of not only an ON component for the first modulation cycle but also an ongoing component for subsequent cycles. Because there is some evidence that the temporal code for AMs in the brain stem is converted to a rate code in the ICC (Frisina 2001; Joris et al. 2004; Langner 1992), we focused our analysis on the spike rate in the ongoing response (although we do not discount the possibility that additional information about AMs is carried in spike timing). For each neuron with an ongoing response, we classified the shape of the rMTF (the tuning function relating spike rate to F_{MOD}) based on the range of F_{MOD} for which the firing rate was >50% of its maximum value. The results for two typical neurons are shown in Fig. 2*A*. For the first neuron, the spike rate of the ongoing response was high for F_{MOD} <150 Hz and low for F_{MOD} >150 Hz, yielding a tuning function with a low-pass shape. The spike rate of the second neuron displayed the opposite trend, with relatively low values for F_{MOD} <150 Hz and relatively high values for F_{MOD} >150 Hz, resulting in

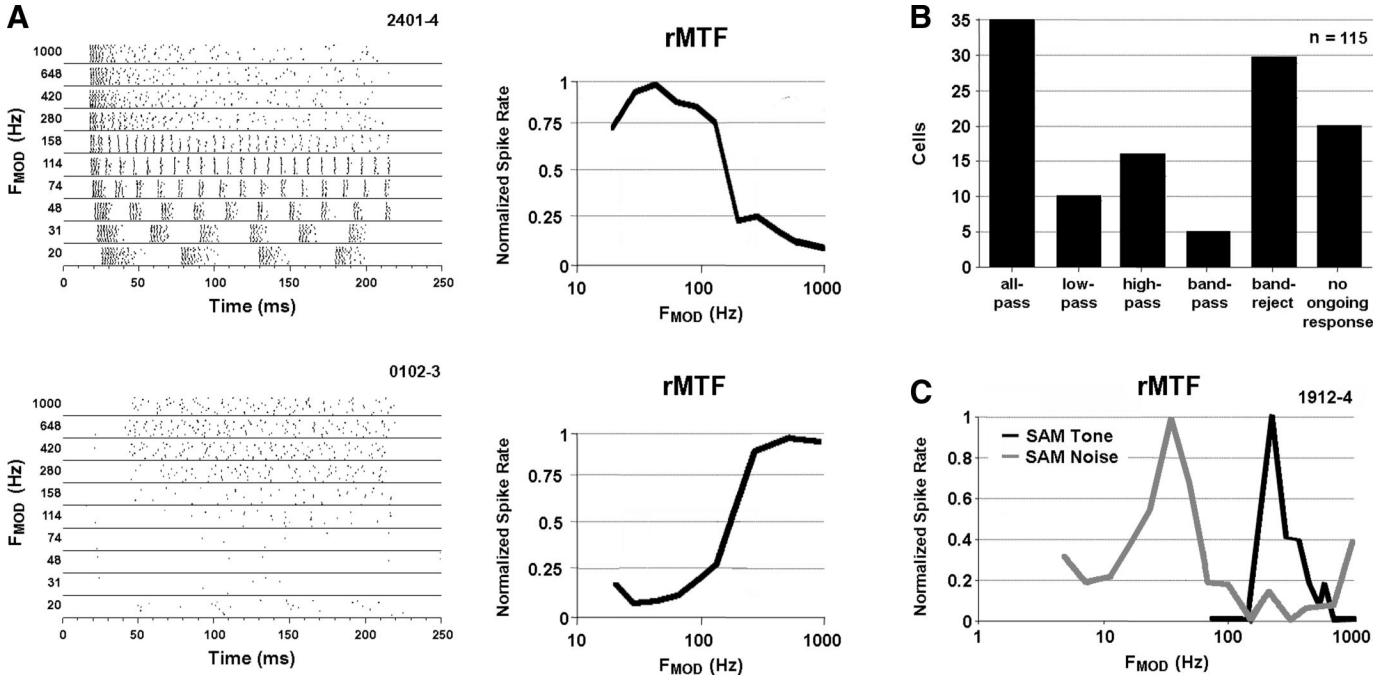

FIG. 2. The responses of inferior colliculus (ICC) neurons to SAM stimuli. *A*: the responses of 2 typical neurons to SAM tone stimuli with different modulation frequencies (pure tone carrier at best frequency, 20 dB above threshold, 100% modulation depth). Raster plots showing the spike responses to 10 repeated trials and rate modulation tuning functions (rMTFs) showing the average effect of modulation frequency on the ongoing spike rate (normalized to the maximum value). The maximum spike rate evoked by the SAM tone stimuli was 160 Hz for neuron 2401-4 and 86 Hz for neuron 0102-3. *B*: the distribution of tuning function shapes based on the range of modulation frequencies where the response exceeded 50% of the maximum value for a population of 115 cells. *C*: the rate modulation tuning function (rMTF) showing the average effect of modulation frequency on the spike rate of a typical neuron for SAM tone and SAM noise (gaussian white noise carrier, 20 dB above threshold, 100% modulation depth) stimuli. For this neuron, the maximum spike rate evoked by the SAM tone stimuli was 112 Hz, and the maximum spike rate evoked by the SAM noise stimuli was 97 Hz.

a tuning function with a high-pass shape. Across the population, we observed a diversity of tuning function shapes including low-pass (10%, 10/95), high-pass (17%, 16/95), band-pass (5%, 5/95), all-pass (37%, 35/95), and band-reject (31%, 29/95), as summarized in Fig. 2*B*.

For some cells, we also recorded responses to SAM noise stimuli (gaussian white noise carrier, 20 dB above threshold, 100% modulation depth). In 19 cells, we observed an ongoing response to both SAM tone stimuli and SAM noise stimuli. The rMTFs for one of these neurons for both SAM tone and SAM noise stimuli are shown in Fig. 2*C*. The tuning function for SAM tone stimuli (black) has a sharp band-pass shape with the peak at 114 Hz and no response to F_{MOD} <100 Hz. For SAM noise stimuli (gray), the tuning function changes dramatically, with the peak shifting to 31 Hz and only a small response for F_{MOD} >100 Hz. Of the 19 cells with an ongoing response to SAM stimuli with both tone and noise carriers, only 7 (37%) exhibited the same tuning function shape (band-bass, low-pass, etc.) in both cases, whereas the other 12 (63%) changed tuning function shape. These results provide a clear illustration that the relationship between neural responses and F_{MOD} cannot be unambiguously determined from responses to a single SAM stimulus, because changes in spectral energy (or, as described in previous studies, mean level, modulation depth, and background noise level, see DISCUSSION) can result in dramatically different responses to stimuli with the same periodicity.

Responses to pulse train stimuli

To provide a more detailed characterization of the representation of AMs in midbrain responses, we recorded the responses of 37 cells to a series of pulse train stimuli (trapezoidally amplitude modulated tones, pure tone carrier at BF, 20 dB above threshold, 100% modulation depth) with different DURs and IPIs. As shown in Fig. 3*A*, these stimuli contain AMs in which four potentially important parameters are systematically varied: the DUR, the IPI, the DC, and the F_{MOD}. The responses of a typical neuron to SAM tone stimuli and pulse train stimuli are summarized in Fig. 3, *B* and *C*. This neuron displays strong tuning to F_{MOD} in SAM tone stimuli, as evidenced by the low-pass rMTF shown in Fig. 3*B*. The responses of this neuron to the pulse train stimuli with different values for DUR and IPI are summarized in the surface shown in Fig. 3*C*. The largest response is elicited by pulse trains with large values for DUR and IPI, and the direction along which the variance in the response surface is maximal (pink arrow) is oriented midway between the directions corresponding to changes in DUR and F_{MOD} (see Fig. 3*A*). This suggests that this neuron is sensitive to changes in both F_{MOD} and DUR.

To quantify the sensitivity of a neuron to DUR, IPI, DC, and F_{MOD}, we calculated the partial Kendall rank correlation coefficients between the spike rate and each stimulus parameter. The partial correlation coefficient (CC_p) quantifies the effects of each stimulus parameter on the neural response independently, removing any ambiguity that arises caused by covariations between different stimulus parameters. Based on the CC_p to Z transform with Bonferroni correction for a population of 37 cells, only values of $|CC_p| > 0.28$ are statistically significant at the $P < 0.05$ level. According to this criterion, the response of the neuron shown in Fig. 3, *B* and *C,* was sensitive to DUR ($CC_p = 0.72$) and F_{MOD} ($CC_p = -0.75$) and insensitive to changes in IPI ($CC_p = 0.24$) and DC ($CC_p = 0.15$).

The responses of a second neuron to SAM tone stimuli and pulse train stimuli are summarized in Fig. 3, *D* and *E*. This neuron also displays strong tuning to F_{MOD} in SAM tone

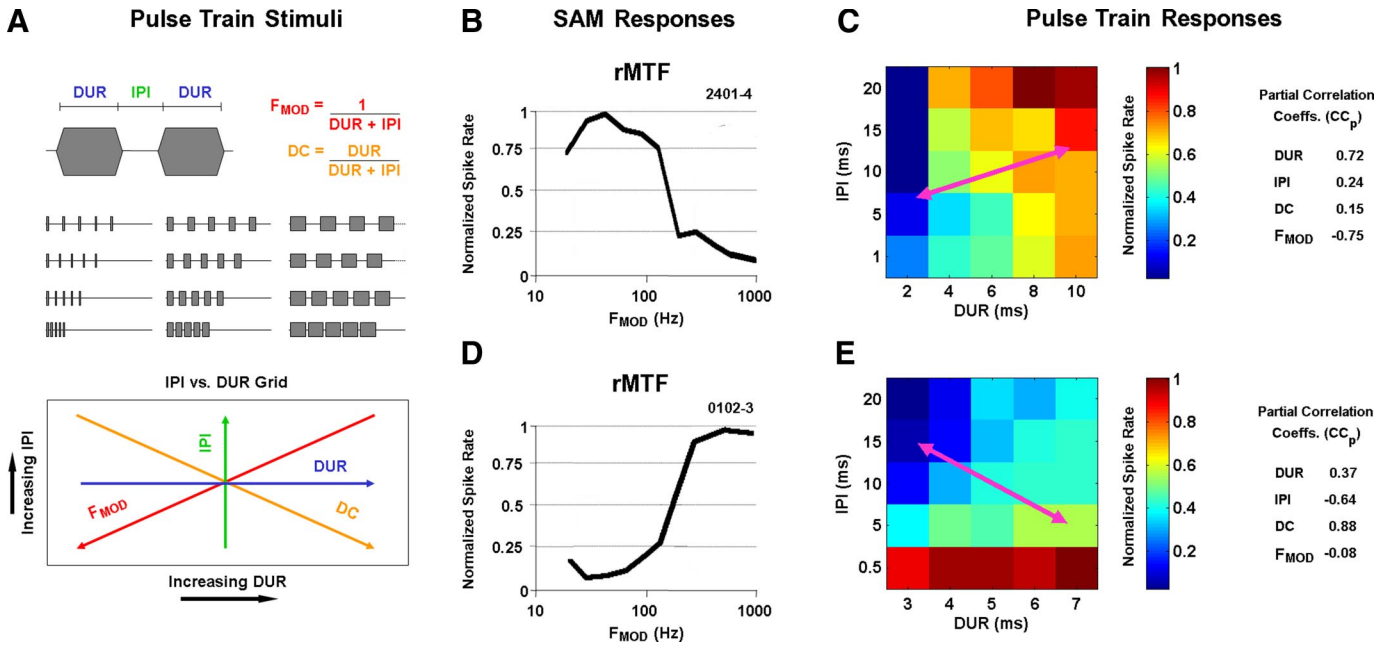

FIG. 3. The responses of ICC neurons to pulse train stimuli. *A*: the schematic diagrams show the changes in DUR, IPI, DC, and F_{MOD} in a series of pulse train stimuli. *B* and *D*: the rMTFs showing the average effect of modulation frequency on the spike rate of 2 typical neurons for SAM tone stimuli (the same neurons as in Fig. 2*B*) *C* and *E*: the response surface summarizing the effect of DUR and IPI on the spike rate of 2 typical neurons for pulse train stimuli (pure tone carrier at best frequency, 20 dB above threshold, 100% modulation depth). The maximum spike rate evoked by the pulse train stimuli was 106 Hz for neuron 2401-4 and 110 Hz for neuron 0102-3. The direction along which the variance in the response surface is maximal is denoted by the pink arrow. The partial correlation coefficient (CC_p) between the spike rate and DUR, IPI, DC, and F_{MOD} for each neuron is also shown.

stimuli, as evidenced by the high-pass rMTF shown in Fig. 3*D*. For this neuron, the largest response is elicited by pulse trains with large values for DUR and small values for IPI, and the direction along which the variance in the response surface is maximal (pink arrow) nearly orthogonal to the direction corresponding to changes in F_{MOD}. As measured by CC_p, the response of this neuron was sensitive to DC ($CC_p = 0.88$), IPI ($CC_p = -0.64$), and DUR ($CC_p = 0.37$) and insensitive to changes in F_{MOD} ($CC_p = -0.08$).

Across the population of 37 cells, we observed a range of sensitivities to the different stimulus parameters, as summarized in Fig. 4*A*. The parameter for which we observed the largest number cells with significant sensitivity was DUR (28/37), followed by DC (20/37), IPI (19/37), and F_{MOD} (17/37). These results suggest that AMs are represented in the responses of individual neurons in the auditory midbrain not only by their periodicity but rather by a combination of several stimulus parameters.

To identify groups of neurons with similar sensitivity for one or more stimulus parameters, we specified the response of each neuron in a four-dimensional space according to its CC_p for DUR, IPI, DC, and F_{MOD} and sorted the neurons in clusters (see METHODS). After sorting, the mean distance from each neuron to the centroid of its assigned cluster (0.26 ± 0.15) was much smaller than the mean distance between the centroids of different clusters (1.22 ± 0.28), and the cluster centroids were significantly different (1-way ANOVA, $P < 10^{-9}$).

The results of the cluster analysis are shown in Fig. 4*B*. We observed two dominant clusters, the first of which (red, $n = 15$) was, on average, most sensitive to DC ($CC_p = 0.47$) and the second of which (green, $n = 14$) was most sensitive to DUR ($CC_p = 0.63$). We also observed two additional small clusters, the first of which (blue, $n = 4$) was most sensitive to F_{MOD} ($CC_p = -0.71$) and the second of which (cyan, $n = 3$) was also most sensitive F_{MOD} ($CC_p = 0.41$). We also observed one outlier neuron (magenta) that was most sensitive to DC ($CC_p = 0.81$). A summary of the sensitivities of each cluster, along with the number of cells in each cluster with significant sensitivity to each parameter are indicated in the table shown in Fig. 4*C*. These results suggest the neural representation of AMs in the auditory midbrain may be organized into distinct functional clusters, each of which is sensitive to a complex combination of stimulus parameters.

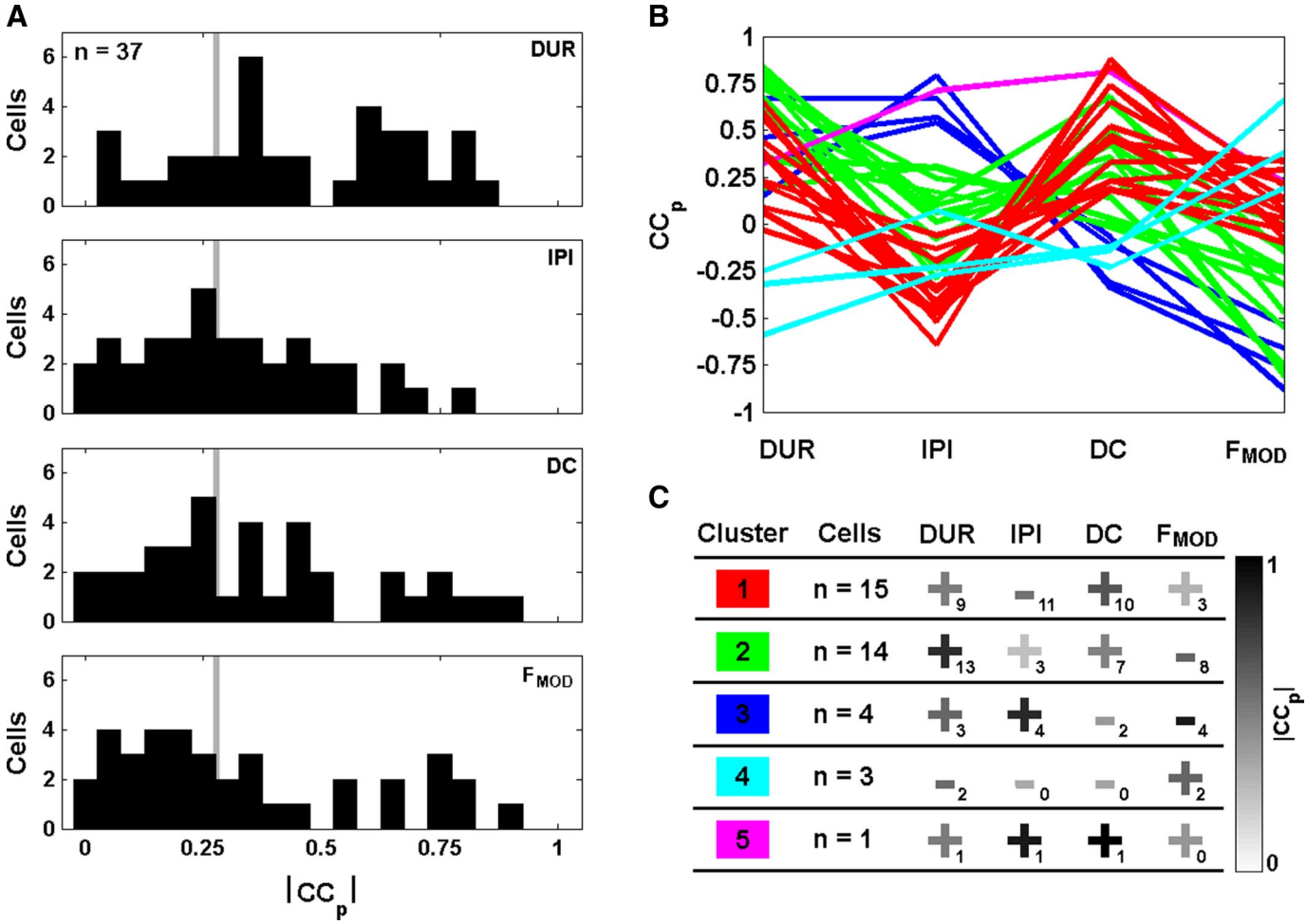

FIG. 4. ICC neurons are sensitive to combinations of stimulus parameters. *A*: the distribution of absolute values of partial correlation coefficients ($|CC_p|$) for DUR, IPI, DC, and F_{MOD} in the responses of 37 ICC neurons to a series of pulse train stimuli. The gray line indicates the threshold for statistical significance a the $P = 0.05$ level after Bonferroni correction with $n = 37$ ($|CC_p| = 0.28$). *B*: a plot showing the CC_p for DUR, IPI, DC, and F_{MOD} for 5 different clusters of neurons. Each line corresponds to 1 cell. Lines are colored according to cluster. *C*: a diagram summarizing the sensitivities of 5 different clusters of neurons to AM parameters. The colors correspond to the lines in *B*. The gray level of the + and − symbols corresponds to the mean CC_p for DUR, IPI, DC, and F_{MOD} for each cluster. The numbers next to each symbol indicate the number of cells in each cluster with significant sensitivity to the corresponding parameter.

Partial correlation coefficients provide a useful way to characterize a neuron's sensitivity to different stimulus parameters with a single value. However, it is important to note that CC_p may underestimate the importance of a particular parameter if the effects of changes in that parameter on the neural response are nonmonotonic. For example, a neuron with band-pass sensitivity to a particular parameter may be highly sensitive to changes in that parameter, but the nonmonotonic relationship between the parameter and the response may result in a low CC_p. To study whether this effect resulted in an underestimation of the importance of periodicity in midbrain responses to AMs, we examined the responses to pulse train stimuli for neurons with band-pass tuning to F_{MOD} in SAM tone stimuli. The results for three typical neurons are summarized in Fig. 5.

For the first neuron, the largest response is elicited by pulse trains with similar values for DUR and IPI, corresponding to an F_{MOD} near the peak of the SAM rMTF (note that the range of values for DUR and IPI are different) and the response surface is relatively monotonic. The response of this neuron was sensitive to DUR ($CC_p = 0.65$), DC ($CC_p = 0.65$), and IPI ($CC_p = -0.42$) and insensitive to changes in F_{MOD} ($CC_p = 0.14$). The response surface of the second neuron, which responded most strongly to stimuli with large values for DUR and IPI, is also relatively monotonic, and its response was sensitive to DUR ($CC_p = 0.72$) and F_{MOD} ($CC_p = -0.77$). For these two neurons, the monotonicity of the response surfaces and the high CC_p for certain parameters suggest that partial correlation coefficient is in fact an appropriate measure of parameter sensitivity.

For the third neuron, the response surface is nonmonotonic, with the largest response corresponding intermediate values of DUR and small values of IPI. The partial correlation coefficients for this neuron indicate that it was sensitive to IPI ($CC_p = -0.41$), DC ($CC_p = 0.52$), and DUR ($CC_p = 0.37$). However, because the response surface is nonmonotonic, it is possible that the measured correlation coefficients underestimate the neuron's sensitivity to certain parameters, including F_{MOD}. Nonetheless, it is clear that this neuron has sensitivity to parameters other than F_{MOD}, because its response varies strongly along those directions corresponding to minimal changes in F_{MOD} (green arrows). Taken together, the examples in Figs. 3 and 5 suggest that, whereas partial correlation coefficients may have the potential to underestimate sensitivity to a certain parameter, in most instances, they provide a useful measure of sensitivity under the stimulus conditions tested in this study.

DISCUSSION

The results of this study showed that neurons in the ICC are sensitive to changes in several parameters of AM stimuli including DUr, IPI, DC, and F_{MOD}. In addition to SAM stimuli, we presented pulse train stimuli in which DUR and IPI (and, consequently, DC and F_{MOD}) were systematically varied. For each neuron, we assessed the importance of each stimulus parameter independently by computing the CC_p between each parameter and the spike rate. In this analysis, a neuron that represents AMs by their periodicity alone would have a high CC_p for F_{MOD} and a small CC_p for the other parameters, indicating that its response is invariant to changes in stimulus parameters that do not result in changes in periodicity. Although we observed a small number of such neurons, the majority of neurons had high CC_p for parameters other than F_{MOD}, typically DUR and DC, indicating that their responses are sensitive to changes in stimulus parameters that may not affect periodicity. These results suggest that AMs may not represented in the auditory midbrain simply by their periodicity or any other single parameter but by complex combinations of several important parameters.

Relation to previous studies

The majority of physiological studies involving AMs have used SAM stimuli (for reviews, see Frisina 2001; Joris et al. 2004; Langner 1992) to show that the responses of many neurons in the ascending auditory pathway are strongly dependent on the frequency of the modulation tone. In this study, in addition to SAM stimuli, we presented pulse train stimuli in which not only F_{MOD} but also DUR, IPI, and DC were systematically varied. Our results showed that many neurons in the auditory midbrain are sensitive not only to F_{MOD} but also to other AM parameters (typically DUR and DC). Based on these results and the complexity of AMs as an acoustical property, any conclusions about the representation of AMs in neural responses based on SAM stimuli alone must be carefully evaluated.

Our study is not the first to show that neurons in the auditory midbrain are sensitive to AM parameters other than F_{MOD}. For example, studies in the frog have shown that the responses of some neurons in the torus semicircularis are sensitive to DC (Alder and Rose 2000) and DUR (Gooler and Feng 1992), and studies in the in ICC of bats and mice have shown neurons that are sensitive to DUR (Brand et al. 2000; Casseday et al. 1994; Fremouw et al. 2005). Furthermore, if AMs were indeed represented in the auditory midbrain simply by their periodicity, responses to AM stimuli would be invariant to changes in stimulus parameters that did not affect F_{MOD}. In this study, we showed that many neurons in the ICC displayed different tuning to F_{MOD} in SAM tone and SAM noise stimuli, indicating that the response to AMs is dependent on the spectral energy of the carrier signal. Spectral energy is only one of many examples of stimulus parameters unrelated to periodicity that can cause changes in tuning to F_{MOD}; others include mean level, modulation depth, background noise level, and spatial position (Koch and Grothe 2000; Krishna and Semple 2000; Rees and Palmer 1989).

Technical considerations

Within the ICC at least two distinct subsets of neurons can be distinguished: elongated cells with dendritic trees mainly residing within a single frequency lamina and disc shaped cells with dendrites crossing several frequency laminae (Kuwada et al. 1997; Peruzzi et al. 2000). Additionally, different ICC neurons have distinct intrinsic response properties, with current injections evoking different levels of ongoing spike activity (Peruzzi et al. 2000) and hyperpolarizing currents with different time courses (Koch and Grothe 2003). Thus the possibility that we recorded from some subtypes of ICC cells and missed others that are specialized for periodicity coding has to be considered. Such a bias could be caused by a specific type of recording electrode. However, this is unlikely because we used both glass pipettes and tungsten electrodes. Another possibility is that a bias in the stereotaxic approach led us miss a specific area in the ICC that is sensitive to periodicity. This is also unlikely, not only because we recorded from widely separated electrode tracks in single animals, but also

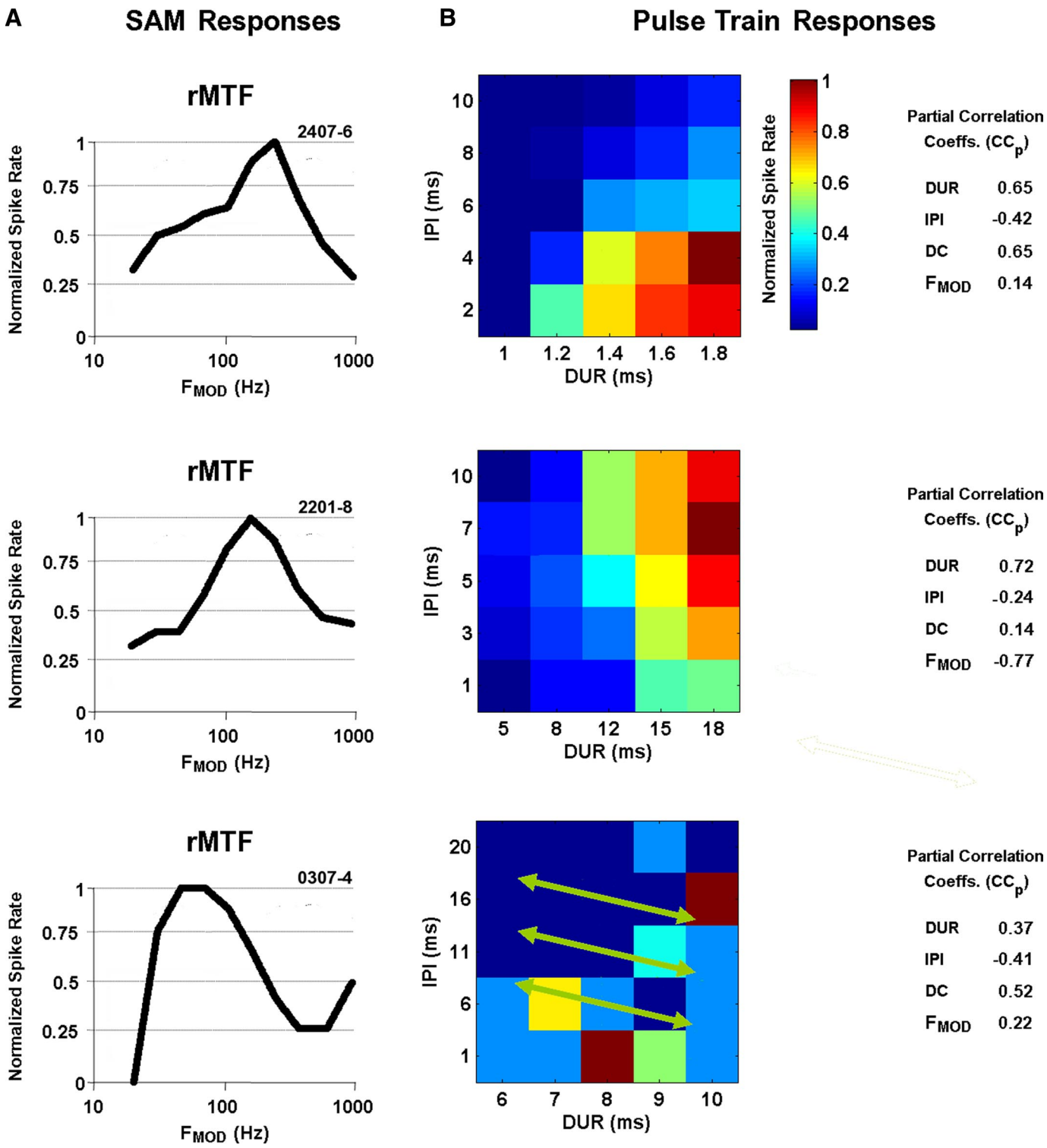

FIG. 5. Responses to pulse train stimuli for ICC neurons with band-pass tuning for modulation frequency in SAM stimuli. *A*: the rMTFs showing the average effect of modulation frequency on the spike rate of 3 neurons with band-pass tuning for F_{MOD} in SAM tone stimuli. The maximum spike rate evoked by the SAM tone stimuli was 125 Hz for neuron 2407-6, 140 Hz for neuron 2201-8, and 8 Hz for neuron 0307-4. *B*: the response surface summarizing the effect of DUR and IPI on the spike rate of 3 neurons for pulse train stimuli (pure tone carrier at best frequency, 20 dB above threshold, 100% modulation depth). The maximum spike rate evoked by the pulse train stimuli was 34 Hz for neuron 2407-6, 353 Hz for neuron 201-8, and 10 Hz for neuron 0307-4. For neuron 0307-4, the directions along which changes in F_{MOD} in the pulse train stimuli were minimal are denoted by the green arrows. The CC_p between the spike rate and DUR, IPI, DC, and F_{MOD} for each neuron is also shown.

because we are not aware of any previous studies that periodicity sensitivity in the ICC is restricted to only a limited area. Our results could also be affected by our choice of anesthesia. However, ketamine is commonly used in studies of temporal processing in the auditory midbrain (Krishna and Semple 2000), and a recent study comparing the response properties of neurons in the

ICC of awake and anesthetized gerbils found only small differences (Ter-Mikaelian et al. 2007). The only major difference between the responses of cells in anesthetized and awake ICC reported by Ter-Mikaelian et al. was in the mean firing rate in response to pure tones >500 ms, which is significantly longer than the stimuli used in our study. Other measures, such as first spike latency and vector strength for SAM stimuli, were remarkably similar in anesthetized and awake animals.

Periodicity maps and pitch perception

The observation that neurons in the ICC are sensitive to changes in the F_{MOD} of SAM stimuli has been cited as evidence that AMs are represented in the midbrain by their periodicity (Langner 1992; Langner and Schreiner 1988), and many theoretical models of auditory temporal processing incorporate a bank of unambiguous periodicity filters as an essential component (e.g., Dau et al. 1997a,b; Dicke et al. 2007). Moreover, because there seems to be a topographic arrangement of the preferred F_{MOD} of SAM tone stimuli in the ICC, it has been hypothesized that the midbrain is organized according to a "periodicity map" (Langner 1992; Schreiner and Langner 1988).

The idea of a periodicity map in the auditory midbrain is attractive for several reasons. First, it implies that the representation of periodicity in the responses of individual neurons is invariant to changes in other stimulus properties, which correlates nicely with the perceptual invariance of pitch to changes in, for example, spectral energy and phase (Houtsma and Smurzynski 1990; Schouten 2008; Seebeck 1841). Second, a topographic arrangement of periodicity allows for simple "read out" downstream. Although the importance of periodicity in responses to AMs is indisputable, our results suggest that the representation of periodicity in the auditory midbrain is not invariant to changes in other AM parameters. However, although the representation of periodicity in individual neurons in the midbrain may be variable, the possibility that an invariant representation of periodicity is created de novo in the cortex cannot be excluded (Langner et al. 1997; Schulze and Langner 1997a,b; Schulze et al. 2002).

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