Temporal Dynamics of Acoustic Stimuli Enhance Amplitude Tuning of Inferior Colliculus Neurons

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INTRODUCTION

Real-world acoustic signals, such as vocal communication signals of various animals, including speech by humans, comprise a large number of sound elements the amplitude and frequency of which change with time. Physiological responses of single neurons at the upper levels of the auditory system have been shown to be dependent on the spectra as well as the temporal ordering of sound components (Doupe 1997; Glass and Wollberg 1983a,b; Margoliash 1983; Steinschneider et al. 1982; Suga 1984; Wollberg and Newman 1972). In the time domain, a unit’s response to a sound is dependent on the rate of stimulation. In general, the selectivity was greater at high rates than at low rates. For a small population of IC neurons, however, the rate of stimulation had little or no effect on their rate-level functions. Thus for IC neurons, responses to sounds presented at low rates may or may not be used to predict the responses to the same stimuli presented at high rates or in a behavioral context. The possible neural mechanisms underlying the rate-dependent effects are discussed.

METHODS

Preparation

Experimental subjects comprised 17 little brown bats, (Myotis lucifugus) weighing 7–10 g. To prepare for electrophysiological recording, the animal was anesthetized via halothane inhalation (4% halothane administered by a precision vaporizer). A custom-made small metal rod then was glued to the skull using glass ionomer cement. After the surgery, the animal was allowed to recover for 2–4 days in an individual holding cage.

Recording procedures

Recordings were made from awake bats. During a recording session, the animal was placed in an open-ended flexible plastic tube inside a sound-attenuating chamber (Industrial Acoustics); the chamber walls are coated with 3-in anechoic foam. The rod that was glued to the bat’s head was secured to a small holder for restraining the animal’s head atarmatically, leaving the ears unobstructed for free-field acoustic stimulation. A sharpened tungsten wire was used to make a small hole (~50 μm) in the skull overlying the recording area in the IC. Throughout the recording session, the animal was offered drinking water periodically and monitored for signs of discomfort. After a recording session of 6–8 h, the exposed skull was covered with sterile bone wax, and the animal was returned to its holding cage.
If the bat struggled or showed signs of restlessness, the recording session was terminated. Such experiments proceeded every 2–3 days for a maximum of 3 wk.

Extracellular single-unit recordings were made with glass micropetites (10–20 MΩ, 2 to 3-μm tip) filled with horseradish peroxidase [5% in 0.2 M tris(hydroxymethyl)aminomethane buffer]. Such electrodes produce stable recording over long duration and allow marking of the recording loci (no marking was carried out in this study, however), and thus were preferred over other types of recording electrodes. The electrode was positioned above the IC by means of a precision (1 μm) digital micromanipulator and lowered to the dorsal brain surface. The relative position of each electrode was monitored from the readouts of digital micrometers using a common reference on the skull. Vertical advancement of the electrode was made by a remote-controlled hydraulic microdrive (Kopf 1207S) from outside the sound-attenuating chamber. Recorded action potentials were monitored audiovisually, amplified with 2400 extracellular amplified (DAGAN) and stored on a video recorder (Vetter model 400), and processed off-line (100 μs binwidth) with data-acquisition software/hardware from RC Electronics.

The animal quarters are inspected regularly by the veterinary staff and the University of Illinois Lab Animal Use and Care Committee. Our experimental protocols were reviewed and approved by the University of Illinois Lab Animal Use and Care Committee and are in compliance with the “Guide for the Care and Use of Laboratory Animals” Publication No. 86-23 (revised 1985) of the National Institutes of Health and with the Animal Welfare Act of 1966 and its amendments of 1970 and 1976.

Acoustic stimulation

Tone pulses of 2-ms duration (including a rise-fall time of 0.5 ms), ranging in amplitude from 0 to 90 dB SPL in 2-dB increments, were presented in three different configurations (Fig. 1; see RESULTS for rationales). The first was individual tone pulses of constant amplitude presented at different pulse rates from 1 to 100 pulses/s (pps)—these shall be referred to as constant-amplitude tone pulses (Fig. 1A). At each pulse rate, to construct a unit’s RLF at that pulse rate, the amplitude of tone pulses was increased systematically with steps of 2 dB over the 90 dB range. The second comprised random-amplitude pulse trains, i.e., long continuous trains of tone pulses presented at a fixed pulse rate (ranging from 1 to 100 pps) of the amplitudes which were ordered randomly (Fig. 1B). At each pulse rate, the random pulse train was divided into 10–20 blocks of 46 tone pulses having a uniform distribution of pulse amplitudes from 0 to 90 dB SPL in 2-dB steps with each pulse amplitude presented just once within a block. Thus each pulse amplitude appeared 20 times in a train of 20 blocks. The third was dynamic-amplitude-modulated pulse trains, i.e., short (625 ms) trains of tone pulses the pulse rate of which increased progressively from 20 to 100 pps, approximating the temporal sequence of sonar emission during a target pursuit (Fig. 1C). The amplitude of tone pulses was sinusoidally amplitude modulated (AM) at a fixed frequency, from 5 to 110 Hz, covering the range of wing-beat frequencies of the bat’s natural prey. Sinusoidal modulation represents a first approximation of insect’s wing beat pattern. The AM pattern of such stimuli thus resembles what bats encounter during an insect pursuit (Kober and Schnitzler 1990; Moss and Zagaeski 1994; Schnitzler et al. 1987). Each dynamic-AM pulse train was repeated 10–20 times, once every 2 s, to obtain sufficient samples for construction of unit’s RLF.

Unless specified otherwise, tone pulses were presented at the unit’s characteristic frequency (CF). Although little brown bats employ frequency-modulated (FM) sound pulses during echolocation, tone pulses were chosen for practical reasons. Foremost, tone pulses are well characterized in terms of its spectrum and time course and represent standard stimuli used in physiological study of the auditory system. Importantly, tone bursts are especially well suited for characterizing unit’s rate-level function and frequency response area. Frequency-modulated stimuli are highly complex by comparison. A unit’s response to a FM stimulus depends on complex interactions between the stimulus level, the sweep direction and rate, and the initial and final frequencies (Gordon and O’Neill 1998). There are thus too many stimulus parameters that can influence the strength of a unit’s response.

Sounds were delivered to the bat via a free-field ultrasonic loudspeaker (Ultra Sound Advice US-LS) located 60 cm in front of the bat. The absolute peak sound pressure level was measured with a ¼-in microphone (Brueel and Kjaer 4135) situated near the concha of the ear opposite to the recording side. Acoustic stimulus parameters were controlled by D/A hardware and software from Tucker-Davis Technologies.

Data analysis

For all three types of stimuli, the unit’s response to each of the tone pulses was used to construct its rate-level function. For each type of stimuli, 10–20 tone pulses at each individual pulse amplitude were presented, and this number was identical across all amplitudes. The average spike count, instead of the average firing rate, was used as the response metric because some IC neurons only fired one spike per tone pulse. The mean latency of the first spike within the response window (see following text) was taken as the unit’s response latency to a particular sound pulse. In the case of constant-amplitude tone pulses, at each pulse rate the stimuli were presented in sets (see Fig. 1A for 1 set), beginning from low sound levels and progressing to higher sound levels in incremental steps. In the case of dynamic-AM pulse train, to identify the tone pulse in the train to which the unit responded, we compensated for the delay in response latency during off-line analysis by an amount equivalent to the average latency to
constant-amplitude tone pulses. Action potentials falling within a 7-ms window relative to the onset time of a tone pulse (i.e., average latency ±3.5 ms) were defined as the response to that pulse. We found empirically that the maximum fluctuation in response latency due to a change in pulse rate was on average 1.12 ms and always <2.1 ms (Fig. 4). As such, the chosen time window was long enough to reliably identify the tone pulse in the sequence to which a neuron responded, in spite of the fluctuations in response latency. The identification of the tone pulse that elicited a response was calculated automatically by custom-made software (written by Ken White and Olga Galazyuk).

**Comparison of experimental data with adaptation model**

An increase in rate of stimulation sometimes led to an overall decline in the firing of IC neurons. To distinguish between nonspecific response adaptation caused by high stimulation rates and rate-dependent selective suppression, neuronal responses to tone pulses within a train were compared with simulated neuronal responses generated by a simple numerical model containing synaptic adaptation (Eggermont 1985, 1999; Valera et al. 1997). The model assumes that the probability of a response of a neuron to the Nth pulse in a train is dependent on the amplitude of the Nth tone pulse, the interval between the Nth pulse and the preceding tone pulse, the amount of depression caused by the preceding tone pulse, and the recovery time constant of the neuron. The adaptation effects are cumulative, yielding the following relationship

\[
P(N) = \prod_{n=1}^{N} A(N) \cdot (1 - d(N)) \cdot \exp\left(-\left((N - n + 1) \cdot \Delta t\right)/\tau\right)
\]

where \(P(N)\) = probability of a response to the Nth tone pulse in the sequence; \(A(N)\) = amplitude of the Nth tone pulse in the sequence; \(d(N)\) = fraction of depression caused by the previous tone pulse in the sequence, where \(d(N)\) is a function of \(A(N-1)\), with \(d(1) = 0; \Delta t = \) interpulse interval (in ms); \(\tau = \) recovery time constant (in ms).

This model lumps all biophysical properties of real neural networks into a single parameter, \(\tau\). Simplified schemes such as this have been used successfully to describe the adaptation processes in the peripher-\al auditory system (Eggermont 1985), auditory cortex (Eggermont 1999), and visual cortex (Varela et al. 1997).

The behavior of the adaptation model was compared with the responses of 16 neurons displaying rate-dependent decreases in amplitude tuning. The model was presented with sequences of constant-amplitude pulses at 1, 10, 20, 50, and 100 pps, similar to the stimuli used during the physiological experiments (Fig. 1A). For each comparison, the output of the model in response to constant-amplitude pulses at 1 pps was matched to the output of the neuron being modeled. At higher rates, the parameter \(\tau\) of the model was adjusted so that the peak output of the model, in terms of maximal spike output, matched the peak output of the neuron at any individual stimulation rate (see Fig. 9). This was done to allow direct comparison of the output of the model to the responses of the neuron at any particular pulse rate. This ensures that any differences in the AR between model output and neuronal response could not be attributed to changes in the overall responsiveness of the neuron. Model and real unit selectivity to amplitude was assessed by determining the width of the rate-level function at 50% of the maximum output of the neuron at any particular pulse rate.

**Results**

RLF data were obtained from 149 neurons in the IC of *M. lucifugus*. In this study we made a conscious attempt to sample neurons from a broad area within the inferior colliculus and across all frequency bands. In agreement with Condon et al. (1994), the CFs of these neurons ranged from 7 to 80 kHz, encompassing the frequency range of the little brown bat’s echolocation signals, as well as the species social communication signals (Dalland 1965). The threshold at CF ranged from 3 to 92 dB SPL, with a modal value of 52 dB SPL. Units with low CFs were represented in the dorsal aspect of the IC and higher frequencies more ventrally within the nucleus.

**Responses to constant-amplitude tone pulses**

Responses of 79 IC neurons to constant-amplitude tone pulses (Fig. 1A) were studied. For each unit, we constructed its RLFs at different rates of stimulation, from 1 to 100 pps. For 28% of IC neurons (27/79), their responses to tone pulses were critically dependent on rate of stimulation as reported previously (Condon et al. 1994; Pinheiro et al. 1991). Specifically, many of these neurons did not respond to tone pulses presented at 1 pps, but responded to tone pulses only when the pulse rate was higher. Others were the opposite; these cells responded well to tone pulses at low pulse rates but showed no response when tone pulses were presented at higher rates. Because of the extreme selectivity to rate of stimulation, the unit’s RLF could not be derived and analyzed quantitatively at the different pulse rates presented. Consequently we could not quantify the effect of rate for these neurons.

The majority of IC neurons (\(n = 57\)), however, responded well to tone pulses presented at various different rates of stimulation. At 1 pps, these neurons either exhibited monotonic, nonmonotonic, or multipeaked RLFs (Fig. 2, A–C, top; also D and E, top). For about one-half of this population (27/57), irrespective of the shape of the unit’s RLF, an increase in rate produced a conspicuous sharpening of its RLF. The increase in amplitude tuning was attributed to a selective drop in spike counts mainly at low and high sound levels (Fig. 2, A–C). For the unit in Fig. 2A, its RLF was transformed from saturating-monotonic at 1 pps to increasingly nonmonotonic at higher pulse rates. At 100 pps, this unit showed a sharp amplitude selectivity with a best amplitude (BA) of 66 dB SPL. The IC unit in Fig. 2B had a nonmonotonic RLF at 1 pps. For this unit, an increase in rate produced a progressive sharpening of amplitude tuning. The unit in Fig. 2C exhibited a multipeaked (or complex) RLF at 1 pps. Similar to the other two IC units, an increase in rate elicited suppressions of the response at low (<20 dB SPL) and high (>30 dB SPL) sound levels. As a result, the multipeaked RLF was transformed into a simple nonmonotonic RLF at 100 pps with a BA of 24 dB SPL. For the remaining 30 IC neurons, rate-induced sharpening of amplitude tuning was either less conspicuous or absent (Fig. 2, D and E). As with the other half of IC population, a rate increase progressively reduced the unit’s responses to tone pulses, but the decline in response (i.e., the unit’s sensitivity) was fairly uniform over the broad range of sound levels investigated (including the response at the unit’s BA). As a result, the shape of unit’s RLF was unchanged. Although the RLF shape was unchanged, a near uniform decrease in response sometimes could also give rise to an increase in amplitude selectivity (Fig. 2D).

To evaluate how rate of stimulation influenced the amplitude selectivity quantitatively, we measured the width of the amplitude response ranges (ARs) at different rates. For each rate of stimulation, AR was defined as the range over which the...
response was equal to 50% below the maximum spike count at that rate. With a few exceptions (n = 5), an increase in rate produced a progressive decrease in AR for all IC neurons within this population (Fig. 3A). The amount of change in AR was highly variable, with most showing the largest decrease at 50 pps. The maximum reduction in the AR between 1 and 50 pps was 40 dB.

As noted previously, a widespread rate-induced decline in spike counts, i.e., a reduction in sensitivity over a broad range of sound levels, also could lead to narrowing of amplitude tuning (Fig. 2D). To determine the extent to which narrowing of the AR was attributed to a decrease in sensitivity, versus a change in selectivity, we measured the normalized AR and how it changed with rate of stimulation. For this, we normalized the RLFs at all pulse rates to the response peak of the RLF at 1 pps, and from these we obtained the normalized ARs. As shown in Fig. 3B, for 30 of 57 neurons, the normalized AR showed an increase (n = 21) or was essentially constant (n = 9), instead of a decrease, with rate. This result indicated that the decrease in absolute AR observed previously for these neurons largely was attributed to a decrease in sensitivity (see example in Fig. 2E).

In contrast, for 27 IC neurons, both the absolute AR and the normalized AR were progressively reduced with an increase in rate of stimulation. For these neurons, there was a selective decrease in response at low and high sound levels with only a slight reduction in the unit’s response at the BA. The decreases in both ARs reflected a genuine increase in amplitude selectivity. Taken together, an increase in rate of stimulation produces a sharpening of amplitude selectivity for about one-half of IC neurons.

**FIG. 2.** Rate-level functions of 5 representative inferior collicular (IC) neurons to constant-amplitude tone pulses presented at different pulse rates. One population of IC neurons exhibits a narrowing of the rate-level function (RLF) with an increase with pulse rate (A–C). Another population of IC neurons shows primarily a lowering of the RLF with an increase in pulse rate (D and E). A: neuron showing a monotonic RLF when stimulated at 1 pps (top). An increasing in pulse rate transforms its RLF from monotonic to nonmonotonic (bottom panels). B and C: neurons showing nonmonotonic and multi-peak RLFs at 1 pps. At 100 pps, these RLFs are transformed into sharp nonmonotonic and single-peak nonmonotonic RLFs, respectively. D and E: IC neurons with nonmonotonic and monotonic RLFs (respectively) exhibit lowering of the spike count when the rate is increased.

**FIG. 3.** Effect of rate of stimulation on the absolute amplitude response range (AR; A) and normalized AR (B) for 48 IC neurons with reference to the AR to tone pulses presented at 1 pps. See text in the paper for derivation of the normalized AR.
FIG. 4. Effects of rate on unit’s response latency. Shown here are data from 49 IC neurons for which latencies were measured in response to constant-amplitude tone pulses presented at rate of 1, 10, 20, 50, and 100 pps. For each unit, response latency was normalized to the response latency measured at the rate of 1 pps. • mean value with SD at given pulse rate. For the majority of IC neurons, the response latency progressively increased with pulse rate.

The CFs of neurons exhibiting rate-dependent sharpening of its RLF ranged from 23 to 86 kHz with a modal value of 47 kHz. This corresponded to the range of frequencies of echolocation signals for little brown bats. However, because the frequency range of bat’s social communication signals overlap with that of sonar signals (Fenton et al. 1976), it is unclear whether this phenomenon is associated strictly with echolocation.

Effects of pulse rate on response latency

In agreement with previous studies in bat IC (Jen and Chen 1998) and cat auditory cortex (Phillips et al. 1989), the response latency was lengthened progressively as the pulse rate was increased (Fig. 4). The mean response latency at 1 pps was 10.6 ± 2.4 ms (mean ± SD; range = 7–17.5 ms). This value increased by an average of 0.26, 0.53, 1.12, and 2.1 ms when the pulse rate was increased to 10, 20, 50, and 100 pps, respectively. To determine if the change in response latency was statistically significant, we performed logarithmic regression analysis of the averaged data. Logarithmic regression analysis was chosen because there was an increase in both the mean as well as the standard deviation with rate. The slope of the regression was 0.246. This slope was significantly greater than zero (P < 0.00001, 1-tailed t-test), indicating that the change in response latency was significant.

There was no correlation between rate-induced changes in amplitude selectivity and response latency. For example, some neurons showing a drastic increase in amplitude tuning with pulse rate did not exhibit a change (or only a minute change) in their response latency. Other neurons showing a marked increase in response latency with pulse rate exhibited little or no increase in amplitude tuning.

Responses to tone pulses in random-amplitude pulse trains

To determine whether or not the systematic ordering of amplitude of constant-amplitude tone pulses (Fig. 1A) was important for the changes in RLF, we investigated and compared the responses of 28 IC neurons to random-amplitude pulse trains and to constant-amplitude tone pulses at fixed repetition rates. We found that, for all neurons, the shapes of RLFs as derived from the two stimulation paradigms were very similar, as exemplified by the results from one neuron (Fig. 5). For this neuron, an increase in rate (≤50 pps) elicited a systematic sharpening of the RLFs with both types of stimuli.

To test whether the RLFs derived from the two types of stimuli (at the same pulse rate) were statistically different, we performed a least-square regression analysis of RLFs with the two stimulation paradigms. Because the pulse amplitudes (the x values in Fig. 5, A and B) were identical for both stimulation paradigms, we essentially tested whether or not the spike counts (y values in Fig. 5, A and B) were significantly different.

For this, we plotted the responses to tone pulses in random-amplitude pulse trains against the responses to constant-amplitude tone pulses at the same pulse rate (see Fig. 5C). We first performed a least-square linear regression of each data set and determined the slope of the regression line and its intersect with the ordinate. The regression data from all 28 neurons were pooled. We found that the average slope was 0.84 ± 0.1. This slope was not significantly different from the slope of 1 (2-tailed Student’s t-test: P = 0.54), indicating that the two sets of data were highly correlated. The average intercept was 0.19 ± 0.31, and this was not significantly different from 0 (2-tailed Student’s t-test: P = 0.64). These results showed that differences in the shape and in the response magnitude of the RLFs were not statistically significant.

The preceding results additionally suggested that the ordering of tone pulses was not particularly important for the increase in amplitude selectivity and that the rate of tone pulses was the most important determining factor. This implied that the response of IC units to a tone pulse at BA was independent of the amplitude of the preceding tone pulse. To test this hypothesis further, we have analyzed the distribution of amplitudes of tone pulses in the random-amplitude pulse trains that preceded the tone pulses to which IC neurons responded (i.e., around a unit’s BA). We found that the distribution of amplitudes of the preceding tone pulses was broad and that the preceding tone pulse could be either weaker or more intense than the BA pulse (Fig. 6). For IC neurons having a low BA (Fig. 6A, V), the tone pulses preceding the BA pulse were mostly of higher amplitudes. For units with high BAs, the majority of the preceding tone pulses were of lower amplitudes (Fig. 6C). For units with intermediate BAs, the distribution of amplitude of the preceding tone pulse was broad, encompassing equally lower and higher amplitudes (Fig. 6B).

Responses to dynamic-AM pulse train

In natural situations, when bats pursue a target they change the rate of sonar emission dynamically, and thus the rate of echoes that they must analyze is also dynamic. To investigate whether the rate-dependent changes in RLF were applicable in a behavioral context, we investigated in 46 neurons the unit’s responses to dynamic-AM pulse train and to control stimulus (i.e., constant-amplitude tone pulses at 1 pps). As described in METHODS, pulse rate in the dynamic-AM pulse train increased exponentially with time from 20 to 100 pps, and sound pulses within the train were sinusoidally amplitude modulated at fixed AM frequencies. Each neuron was tested with four or more dynamic-AM pulse trains each at a different AM frequency; note that a change in AM frequency produced a change in temporal orders of sound pulses.
For 21 neurons (47%), the RLFs in response to tone pulses embedded in dynamic-AM pulse trains were sharply nonmonotonic, whereas the unit’s RLF to constant-amplitude tone pulses at 1 pps was either broad nonmonotonic (15 units; Fig. 7, A–D) or monotonic (6 units; Fig. 7, E–H). These cells displayed sharp amplitude selectivities when presented with stimuli the temporal patterns of which approximated what bats experience while hunting, in spite of the fact that such tuning actually represented the average of responses at different rates from 20 to 100 pps. As shown by the examples in Fig. 7, these two units responded well to isolated tone pulses at 1 pps over a wide range of amplitudes. In contrast, these units responded to tone pulses in the train only when these pulses fell within a very narrow range of sound levels within a few dB range. Such response selectivity was not sensitive to changes in the temporal ordering of sound pulses within the dynamic-AM pulse train. For both units, the best pulse amplitude was stable, independent of the AM frequency of dynamic-AM pulse trains (compare RLFs in B and D and in F and H). The selectivity was evident near the beginning of the pulse train when the absolute rate was lower or at other segments of the train when the rate was higher (Fig. 7, A and C). The difference in AR between the control and dynamic-AM pulse train thus was attributed primarily to the difference in rates (1 pps for constant-amplitude tone pulses vs. 20–100 pps for the dynamic-AM pulse train). The dynamic-AM data also showed that the number of tone pulses required to induce sharp amplitude selectivity could be as low as two or three (in the beginning of sequence in Fig. 7, E and G).

For the remaining 25 units of the sample, there was either no distinct difference in the RLFs for the two types of stimuli (n = 21) or there was a dramatic drop in spike count in the presence of the dynamic-AM pulse train (n = 4).

**BAs**

Different IC neurons responded best to different BAs. The data from constant-amplitude tone pulses and the dynamic-AM pulse train showed that the BAs were distributed more or less uniformly over the range of 5–85 dB SPL (Fig. 8). Thus although each neuron was sharply tuned to a narrow range of amplitude, the wide distribution of BAs assures that bats would have high resolutions across the broad range of sound levels encountered during echolocation.

**Adaptation versus selective suppression**

To evaluate whether or not, and the extent to which, rate-dependent sharpening of amplitude selectivity was attributed to adaptation, we carried out a modeling study comparing the output of a simple adaptation model with the output of 16 IC neurons displaying rate-dependent decreases in AR. The data for one such comparison is shown in Fig. 9. By design, the model and real IC neuron being modeled had identical ARs at 1pps (in this case, 51.0 dB). At 10 pps, the model and real IC neuron retained similar AR values (49.5 and 46.1 dB, respectively). However, at higher pulse rates, the adaptation model predicts a slight broadening of the AR, whereas the real IC neuron displayed a sharp drop in the bandwidth; a pattern that is significantly different from the adaptation model. The overall trends are shown in Fig. 10. As the stimulation rate was
increased, the IC neurons showed a decrease in bandwidth that saturated at 50 pps. The adaptation model predicts a gradual increase in bandwidth that also saturates at 50 pps. The broadening of the bandwidth in the adaptation model appears to be due to the ordering of the pulse amplitudes. Because lower amplitude pulses occur earlier in the stimulus presentation sequence (Fig. 1A), responses to lower pulse amplitudes dominate over more intense pulses, and the adaptation primarily weakens the responses to higher pulse amplitude, consequently broadening the rate-level-function. Addition of small amounts of poststimulus facilitation (as used in Eggermont 1999; Varela et al. 1997) did not decrease the RMS error values of the model nor did it promote decreases in AR with increases in pulse rate (data not shown).

**Discussion**

The present study shows that for a large number of IC neurons, certain basic auditory response properties are dependent on the temporal dynamics of acoustic stimuli. Specifically, rate of stimulation can markedly change the RLF of many IC neurons. To the best of our knowledge, this finding has never been described in any detail before. There are several lines of evidence to support this conclusion. First is the direct evidence. We found that for many IC neurons, an increase in rate of stimulation produces a systematic increase in amplitude selectivity; it changes the unit’s RLF from monotonic (or broad nonmonotonic) to highly nonmonotonic. To ascertain that the change in tuning is not simply attributed to a decrease in sensitivity, we have analyzed the data in two ways (i.e., inclusive and exclusive of the change in sensitivity). This analysis reveals that, for about one-half of IC neurons, rate-induced sharpening of amplitude tuning is not due to a change in sensitivity.

We next investigated whether or not, and the extent to which, sequential ordering in stimulus presentation (i.e., ordering of sound pulses in constant-amplitude pulse train; see Fig. 1A) is a contributing factor for the rate-dependent increase in unit’s amplitude selectivity. For this, we compared the RLFs for tone pulses presented in two different manners, in constant-amplitude and random-amplitude pulse trains. We found that the RLFs to tone pulses presented in two pulse trains having very different temporal orders are similar, suggesting that the sequential ordering of tone pulse is not required for the rate-dependent increase in amplitude selectivity.

Finally, results from the dynamic-AM pulse trains provide further supporting evidence for the importance of rate of stimulation in amplitude tuning. Specifically, many IC neurons show highly selective responses to tone pulses in the train that fall within a narrow range of amplitudes. In contrast, their responses to isolated tone pulses are characterized by broad tuning or monotonic RLFs. Sharp amplitude tuning to pulses in the train reflects the difference in the absolute pulse rate (20–100 pps in the train vs. 1 pps for isolated tone pulses). It also is noted that changing the AM frequency of the dynamic-AM pulse train alters the temporal order of these tone pulses in the train and yet this has little influence over the unit’s sharp amplitude selectivity. This result provides further evidence that the temporal order of tone pulses is not particularly important for the amplitude selectivity. Taken together, these data suggest that a change in the pulse rate itself directly influences unit’s amplitude selectivity.

**Implications on forward masking**

The finding that loud as well as weak sound pulses have similar influence over the unit’s amplitude selectivity to a subsequent sound pulse at short interpulse interval has interesting implications on forward masking phenomenon. Forward masking has been observed psychophysically (Luscher and Zwislocki 1947; Stevens and Davis 1938) as well as physiologically at the peripheral and central levels (Brosch and Schreiner 1997; Harris and Dallos 1979). Physiologically, a unit’s response to a probe is weakened when the probe immediately follows a masker, and the response recovers to normal when the masker-probe interval is increased. In most studies, the intensity of the probe is fixed at a suprathreshold level. Our result predicts that physiological manifestation of forward masking may be level dependent for some central neurons.

**Behavioral relevance**

Neural responses to dynamic-AM pulse trains reveal that the increase in amplitude selectivity is evident when the rate changes dynamically. In nature, when a bat pursues a flying insect, its sonar emission is actively increased from 5 to 20 pps...
during the searching phase to 100–200 pps just before prey capture (Griffin et al. 1960; Kalko 1995; Schnitzler et al. 1987). The functional significance of this rate increase has not been pinned down satisfactorily other than the fact that it serves to increase the sampling rate that is useful for tacking the prey at short distance. Our data suggest that overall high rates during target approaching sequence (i.e., higher sonar emission rate and shorter interval between a sonar pulse and its echo) would provide greater amplitude resolution and therefore improves estimates of target size (see following text).

Echo amplitude is correlated with the target size and distance (Griffin 1958; Kick and Simmons 1984). However, during a pursuit, when the emission rate of sonar signals is changed, bats also simultaneously adjust their emission intensities to stabilize the perceived echo amplitude (Hartley 1992; Kick and Simmons 1984; Simmons et al. 1992). As a result, the perceived echo amplitude is relatively independent of the target distance. This maneuver therefore allows bats to use the echo amplitude as reliable information for estimating the target size. Thus in theory, an active increase in rate can presumably confer a higher resolution of target size—this enhancement in

FIG. 7. Responses of 2 IC neurons to dynamic-AM pulse trains having nonmonotonic (A–D) and monotonic (E–H) RLFs. These neurons exhibit marked amplitude selectivities to tone pulses embedded in the dynamic-AM pulse trains (B, D, F, and H, —); they respond only to tone pulses whose amplitudes fall within a narrow range of sound levels (A, C, E, and G, †). Same neurons display monotonic or broad nonmonotonic RLFs to tone pulses presented in isolation at 1 pps (B, D, F, and H, - - -). A, C, E, and G: dot histograms of responses to dynamic-AM pulse trains having a modulation frequency of 20, 50, 5, and 30 Hz, respectively. Tone pulses in the train are shown as vertical bars below each histogram; the height of the vertical bar represents the relative amplitude of the tone pulse within the pulse train.

FIG. 8. Distribution of best sound amplitudes for 48 IC neurons that exhibited sharpening of its RLF in response to constant-amplitude tone pulses (27 units) and dynamic-AM pulse train (21 units) when pulse rate was increased.
sensory capacity is useful for animals that rely on the auditory system to analyze complex auditory scenes. We have found that, at 100 pps, the amplitude tuning width of IC neurons could be as low as 1.4 dB. Amplitude resolution in the range of 1–2 dB would provide a powerful mechanism for precise estimation of target size in the real world.

**Implications for auditory processing**

Our study reveals that some basic response properties of central auditory neurons obtained using tone pulses presented at a low rate (1 pps, which is widely used in auditory research) cannot always predict how these neurons would respond in real-world situations (where sound signals occur in rapid succession). Most auditory physiological studies deliberately have employed sound stimuli at a low rate in order that the unit’s response can be determined accurately without the confounding influence of its firing history. By circumventing this experimental bias, however, the results emerging from these studies may provide only a limited description of the properties of the system, i.e., may only be applicable to tonal stimuli at the rate used in the studies. Our study indicates that generalization of experimental findings may be inappropriate due to the temporal dynamics of the auditory system.

Rate of stimulation previously has been shown to have a variety of effects on higher order auditory processing in different animal models. Wong et al. (1992) reported that rate plays an important role in temporal tuning for neurons in bat’s auditory cortex. Specifically, cortical neurons (77%) are more sharply tuned to the time interval between a pair of sound pulses when the rate of presentation of paired sound pulses is increased. Increasing stimulation rate also sharpens the auditory receptive fields of IC neurons (Wu and Jen 1996).

A rate increase previously has been shown to change certain basic auditory response properties. These changes include increase in the minimum threshold, increase in the response latency (Chen and Jen 1994; Donaldson and Rubel 1990; Jen and Chen 1998; Phillips et al. 1989). Although not specifically stated, the study of Pinheiro et al. (1991, Fig. 10, A–D) also provides illustration of data that are similar to our study. They observed that, for a few IC neurons in a different species of bats, the unit’s RLF changes from monotonic at low rates to nonmonotonic at high rates of stimulation.

Preliminary study in the auditory cortex shows that many cortical neurons also show enhanced amplitude tuning when the rate of stimulation is increased (Galazyuk and Feng 1997). The increase in amplitude tuning seems on the average to be more robust than in the IC. Thus the rate dependent effects appear to be not limited to the IC. At this time, it is unclear whether the rate-dependent effects are applicable to lower centers in the auditory brain stem and to the IC in species other than bats. Clearly this is an issue that deserves further investigation.

Evidence from human psychophysical studies suggests that such sharpening processes occur in the human auditory system. Specifically it was found that a listener’s ability to discriminate the amplitudes of paired pulses is degraded when the interval between two pulses of different amplitude increases from 0.0 to 0.2, 3.5, and 14.0 s (Berliner and Durlach 1972; Berliner et al. 1977). The current study suggests the presence of a physiological basis for such a phenomenon.
Cellular mechanisms

Presently, the cellular mechanism(s) responsible for the rate dependent effects is not clear. A number of factors can influence the response of a neuron to changes in stimulation rate. To distinguish the well-described adaptation effects from other time-dependent response properties, we have compared the performances of two systems, a model system with synaptic adaptation and the other a real IC neuron from the current study. Whereas the adaptation model predicts a small increase in AR with increasing rate at 20 pps, real IC neurons display a nearly 20-dB enhancement in tuning relative to the tuning observed in the adaptation simulations. These findings suggest that although neural adaptation produces an overall drop in neuronal output (as observed with a rate increase), it cannot be responsible for the selective suppression of responses at non-BAs observed in the real data.

We speculate that the time-dependent change in amplitude selectivity may be attributed to either intrinsic mechanism (e.g., biophysical properties of ion channels), or extrinsic mechanism (e.g., synaptic interactions). It is well known that voltage-sensitive K channels are heterogeneous, even at the single cell level and play an important role in stabilizing membrane potential (Hille 1992). Most K channels are opened when the membrane is depolarized, some are activated by membrane hyperpolarization or by cytoplasmic Ca or second messenger. Most importantly, the time course of action varies greatly among K channels. Because of the important role they play in stabilization of membrane potential, they can conceivably regulate a unit’s response threshold in a time-dependent manner.

At the same time, inhibition of unit’s response to intense sounds, as evinced in nonmonotonic RLFs, is a characteristic of central auditory neurons due mostly to synaptic interactions. A number of studies have shown that nonmonotonic RLFs in IC neurons is attributed to GABA-based inhibition (Fuzessery and Hall 1996; Yang et al. 1992; Zhang and Feng 1998). A local application of bicuculline (an antagonist of GABA_A receptor) in the IC can transform nonmonotonic RLFs into monotonic. It is therefore possible that GABA-based inhibition may play a role in the sharpening of units’ amplitude selectivities at high rates. Further research is required to clarify this issue.

We thank D. Goulder for comments on early versions of this manuscript and H. Galfavy (Department of Statistics) for advice on statistical treatment of our data. This study was supported by National Institute on Deafness and Other Communication Disorders Grant RO1-DC-01951. Address for reprint requests: A. V. Galazyuk, Beckman Institute, University of Illinois, 405 N. Mathews Ave., Urbana IL, 61801. Received 25 March 1999; accepted in final form 15 September 1999.

REFERENCES


Zhang, H. and Feng, A. S. Sound direction modifies the inhibitory as well as the excitatory frequency tuning characteristics of single neurons in the frog torus semicircularis (inferior colliculus). *J Comp Physiol [A]* 182: 725–735, 1998.