Effects of Amplitude Modulation on the Coding of Interaural Time Differences of Low-Frequency Sounds in the Inferior Colliculus.
I. Response Properties

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INTRODUCTION

A major cue for determining the location of low-frequency sounds is the interaural time difference (ITD) created by the unequal path lengths that a sound must travel to reach each ear. The ITD responses of inferior colliculus (IC) neurons to tonal stimulation have been extensively studied (Fitzpatrick et al. 1997; Kuwada et al. 1984; McAlpine et al. 1998; Rose et al. 1992; Xu et al. 1996). Van Stokkum and Melssen (1991) found that a large number of midbrain neurons were selective to both ITD and AM, whereas only few were selective for unmodulated tones.

To determine if comparable neurons were present in mammals, we studied the ITD sensitivity of IC neurons to low-frequency tones that did or did not have intensity fluctuations in the unanesthetized rabbit. We found that neurons could simultaneously code the ITD and the AM and that modulation could have a graded effect from extreme enhancement to extreme suppression. The present paper describes these responses in detail, and discusses the possible functional significance. In a companion paper, we investigate the neural mechanisms behind the effects reported here (D’Angelo et al. 2003). Preliminary results were reported in abstract form (D’Angelo et al. 2001).

METHODS

Animals

Single-unit recordings were made from adult, female Dutch-belted rabbits (1.5–2.5 kg) with healthy external ears. All animal procedures were approved by the Committee for Animal Care and Use at the University of Connecticut Health Center and conformed to the guidelines for laboratory animal care and use published by the National Institutes for Health. As surgical and experimental procedures have been fully described elsewhere (e.g., Fitzpatrick et al. 2000; Kuwada et al. 1987), they will be only briefly recounted.

Surgical procedures

All surgery was performed under aseptic conditions. Under anesthesia [ketamine (44 mg/kg) and xylazine (6 mg/kg) im], a square brass rod was anchored to the skull using screws and dental acrylic. About a week later, the animal was again anesthetized to make custom-fitted ear molds. This was done by filling the external meatus and the concha with dental impression material (Reprosil, Dentsply International, Milford, DE). In a separate procedure, a craniotomy (~8 × 4 mm) was performed under anesthesia to allow a dorsal approach to the IC. After each experimental session, the craniotomy was covered with sterilized medical elastopolymer (Sammons-Preston Rolyan, Germantown, IL).

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Recording procedures and data collection

All recordings were conducted in a double-walled, sound-insulated chamber. The unanesthetized rabbit was placed in a spandex sleeve and seated in a padded cradle, and its head was secured to the stereotaxic frame by clamping to the head bar. The elastopolymer was removed, and the topical anesthetic Marcaine was applied to the exposed dura so that penetration by the electrode was painless. Our recording sessions were limited to ~2 h for practical and ethical reasons and could be terminated at any time if the rabbit showed signs of discomfort. Each rabbit participated in daily recording sessions over a period of 3–6 mo. Extracellular recordings were made with glass-coated, tungsten or platinum-tungsten microelectrodes (0.7–1.5 mm tip diameter, 3–15 MΩ impedance). The location of each electrode penetration was determined stereotaxically. The action potentials of single neurons were isolated with the aid of a time/amplitude window discriminator (BAK Electronics, Germantown, MD) and timed relative to the stimulus onset with an accuracy of 10 µs.

Acoustic stimulation

Stimuli were generated using a digital stimulation system (Rhode 1976) and delivered to the two ears through Beyer DT-48 earphones coupled to the ear molds to form a sealed system. The ear molds were fitted with a sound-delivery tube that extended to within ~2.5 cm of the tympanum. The system was calibrated for amplitude and phase from 60 Hz to 40 kHz in 20-Hz steps by means of a probe tube that extended ~1 mm from the end of the sound-delivery tube. Sensitivity to ITDs was assessed by recording the response to a binaural beat stimulus. The first type of binaural beat stimulus was created by delivering tones to each ear that differed by 1 Hz and is schematically illustrated in Fig. 1, A and B. The actual binaural beat is created by the interactions of the neural representations of the signals to the right and left ear (Fig. 1C). This stimulus results in a complete cycle of interaural phase change every second. The second type of binaural beat stimulus was created by adding identical sinusoidal AM to the two ears (Fig. 1, D–F). This resulted in a signal with a continuously varying ITD in the carrier frequency while the envelope ITD remained constant. Natural signals would create a shift in both the carrier and envelope. However, the modulations we typically used have large periods compared with the period of the carrier. So even if we imposed the same ITD shift in the envelope and carrier, the shift in the envelope would be miniscule compared with its period. The peak intensity of the tones and modulated tones were the same (typically 70 dB SPL). Thus the modulated tones had less energy (4.24 dB) than the unmodulated tones.

In the experiments, the two types of binaural beat stimuli were both 5,100 ms long, presented every 6,100 ms (i.e., 5 cycles of the beat frequency). We explored the ITD sensitivity to a range of carrier (200–2,000 Hz) and modulation frequencies (12–400 Hz).

Data analysis

Analyses of neural responses to binaural beat stimuli have been described previously (Fitzpatrick et al. 2000; Yin and Kuwada 1983). We found no significant differences among peak-, trough-, and intermediate-type neurons for the measures described in the following text. Therefore we divided our sample into peak-type and trough-type neurons. Peak-type neurons had characteristic phases between 0.00 and 0.25 cycles or between 0.75 and 1.00 cycles, whereas trough-type neurons had characteristic phases between 0.25 and 0.75 cycles. This analysis was performed only on neurons that displayed significant ITD sensitivity ($P < 0.001$) over at least four frequencies. In the cases where the ITD sensitivity to the unmodulated binaural beat was weak, this classification was based on the response to the modulated binaural beat. Composite ITD curves were obtained by averaging the ITD functions across the responsive frequency range for each neuron using the limitation that the frequencies were matched for the modulated and unmodulated condition. Three measures were extracted from the composite ITD curve: the best ITD, the peak rate, and the ITD tuning width. For each of these measures, a parabolic fit was made to the peak and trough of the composite ITD curve. The best ITD was the center of the parabolic fit to the peak and the peak rate was the rate at the center of the parabola. The peak and trough width of the ITD tuning curve was the width measured 50% between the maximum and minimum response.

If adding modulation to the binaural beat increased the response relative to the unmodulated binaural beat, the neuron was classified as enhanced (positive percent change) and suppressed if modulation decreased the response (negative percent change). This division does not recognize neurons that were relatively unaffected by modulation. However, rather than setting an arbitrary criterion for enhancement and suppression, we simply let the dividing line be zero. Thus our conclusions based on differences between neurons that show enhancement and those that display suppression are highly conservative because both groups contain responses that were not affected by modulation.

To calculate the percent change, the denominator was always the largest response, rather than the response to either the modulated or unmodulated binaural beat. We did so to limit the maximum percent change to +100% and −100% for enhanced and suppressed neurons, respectively. Dividing by the modulated or unmodulated response would create an asymmetrical axis of percent change. More details of our calculation with examples are provided in Results.

Localization of recording sites

During the last several recording sessions, electrolytic lesions (10 µA, 10 s) were made at selected sites where ITD sensitivity was present. Under deep anesthesia [ketamine (44 mg/kg) and xylazine (6 mg/kg) im; pentobarbital sodium (Nembutal; 37.5 mg/kg) iv], the animals were perfused with a 2.5% solution of formal saline. The brains were sectioned and stained with cresyl violet. All of our lesions were located in the IC.
RESULTS

Simultaneous coding of ITD and SAM

We examined the ITD responses to low frequency tones using a 1-Hz binaural beat stimulus with and without sinusoidal AM (SAM) in 272 single neurons from the IC of five unanesthetized rabbits. For a subset of our sample, we examined synchrony (i.e., phase-locking) to different modulation frequencies when added to the binaural beat stimulus (Fig. 2A). This synchrony modulation transfer function (MTF) is low-pass. Because low modulation frequencies produced the highest synchrony and because their sidebands fell within the estimated critical band for our carrier frequencies (300–1,500 Hz), we at least tested the effect of a low modulation frequency (23–25 Hz) on the ITD responses for all neurons.

Almost all neurons synchronized to both the 1-Hz beat and the low modulation frequency. An example of the responses from such a neuron is shown in Fig. 2, B and C. Synchrony is displayed via cycle histograms over a period of the 1-Hz beat frequency (Fig. 2B: contra: 900 Hz, ipsi: 901 Hz, \( R = 0.74 \)) and the modulation frequency (Fig. 2C: 23 Hz, \( R = 0.86 \)). Both responses were significantly synchronized (\( P < 0.001 \)). Note that synchronization to the modulation frequency is also seen as responses at equal intervals within the period of the binaural beat (Fig. 2B). Synchrony to low modulation frequencies (23 and 25 Hz) for our population is plotted in Fig. 2D. Although a few neurons did not synchronize to these modulation frequencies (Fig. 2D, open bar), the vast majority did so (93%) with a modal synchrony near 0.8.

Effect of AM on firing rate

Modulation could enhance or suppress or not affect the discharge rate to the binaural beat. Figure 3 shows the post-stimulus time histograms (PSTs) to the unmodulated (left) and modulated (right) binaural beat for 5 example neurons. In all cases, the stimulus duration was 5,100 ms, and the peak SPL was 70 dB. The examples reflect the responses at frequencies that evoked the maximal peak discharge rate. The effects of modulation followed a continuum (A–E) of extreme enhancement to extreme suppression.
unmodulated and the modulated condition from −100% (suppression) to +100% (enhancement). The difference in the two measures becomes apparent because for this neuron, modulation elevated the response to both favorable and unfavorable ITDs. The unit in Fig. 4B showed the same substantial decrease (−92%) for the peak rate and maximum-minimum measures. The result is similar for both measures because for this neuron, the response to unfavorable ITDs remained unchanged while the response to favorable ITDs was suppressed under modulation.

The distributions of change in peak rate and maximum-minimum measures were similar and occurred for both peak and trough type neurons. Figure 5 shows the changes in peak rate (A) and maximum-minimum (B) measures based on each neuron’s composite ITD curve. We grouped the neurons as enhanced if the change in peak rate was positive and suppressed if the change was negative (Fig. 5A). Clearly, this division does not recognize neurons that were relatively unaffected by modulation. However, rather than setting an arbitrary criterion for enhancement and suppression, we simply let the dividing line be zero. For the peak rate and maximum-minimum measures, 62 and 57% of the neurons, respectively, were classified as enhanced and 38 and 43%, respectively, as suppressed. Although there were more peak- than trough-type neurons, both types were similarly distributed across the enhanced and suppressed groups. Not included in this distribution are the neurons that only responded to the modulated binaural beat (n = 22) and those that only responded to the unmodulated binaural beat (n = 14). More neurons were enhanced for the modulated signal even though it contained less energy.

The frequency tuning of enhanced and suppressed neurons appeared to be similar. We measured the best frequency for 40 enhanced and 42 suppressed neurons. Both types showed a similar distribution (−0.3–16 kHz) with a median best frequency of 2.8 kHz.

To examine the ITD functions of peak- and trough-type neurons that displayed enhancement or suppression, we averaged the ITD functions derived from the unmodulated and modulated binaural beat for the neurons in each category (Fig. 6). As expected, the population ITD functions for peak-type neurons that were classified as enhanced had a higher discharge rate to a modulated versus an unmodulated binaural beat (Fig. 6A, left). Conversely, the population ITD function for suppressed peak-type neurons had a lower discharge rate to a modulated binaural beat (Fig. 6A, right). Interestingly, enhanced neurons displayed about half the peak discharge rate to an unmodulated signal compared with suppressed neurons. This is in agreement with the observation that the response of IC neurons to low modulation frequencies is greatest when the mean discharge rate of the neuron is relatively low (Rees and Palmer 1989). Moreover, the peak rate for the enhanced neurons to the modulated condition was less than that for the suppressed neurons to the unmodulated condition. On the other hand, the peak rate for the suppressed neurons to the modulated condition was higher than the response of the enhanced neurons to the unmodulated condition. When normalized, both enhanced and suppressed neurons displayed highly similar population ITD functions to the unmodulated and modulated signals. The population ITD functions of trough-type neurons paralleled the pattern seen for peak-type neurons (Fig. 6B) except that the degree of enhancement and suppression was less for trough-type neurons (53%) compared with peak-type neurons (75%). Furthermore, to an unmodulated signal, enhanced trough-type neurons displayed only a 31% lower discharge rate than suppressed trough-type neurons, whereas peak-type neurons showed a 57% difference. Finally, the population ITD functions for trough-type neurons (Fig. 6B) did not align as precisely as those for peak-type neurons (Fig. 6A).
Another effect of modulation was to change the frequency range of ITD sensitivity. The ITD frequency range could often be expanded or compressed compared with the unmodulated condition. Figure 8 illustrates these changes and plots the locked discharge rate (synchrony to the binaural beat multiplied by firing rate) versus frequency. A neuron for which the range was expanded (Fig. 8A) displayed ITD sensitivity only at 500 and 700 Hz for the unmodulated condition. With the addition of 25 Hz SAM, the upper range of ITD sensitivity expanded to 1,500 Hz. In contrast, a neuron (Fig. 8B) that showed ITD sensitivity from 500 to 1,500 Hz in the unmodulated condition had a compressed frequency range between 900 and 1,100 Hz for modulation. Finally, we provide an example of a neuron that did not change its range of ITD sensitivity under modulation (Fig. 8C).

For a majority of the units for which the ITD-sensitive frequency range was tested \( (n = 165) \), the range was either expanded (35%) or compressed (40%) and only 25% remained unchanged (Fig. 9A). The expansion or compression of the ITD frequency range was related to whether a neuron’s rate was

![Figure 6](image-url) - The averaged interaural time difference (ITD) functions, i.e., the population ITD function, for peak-type (A) and trough-type (B) neurons further separated for enhanced (left) and suppressed (right) neurons. Top: discharge rate plotted as a function of ITD. Bottom: top normalized to maximal rate.

![Figure 7](image-url) - The distribution of peak firing rate to the unmodulated binaural beat separated for enhanced (■) and suppressed (□) neurons.

![Figure 8](image-url) - Three examples of the effect of modulation on a neuron’s frequency range for interaural time difference sensitivity. The response is plotted as the product of synchrony and discharge rate. Depicted are neurons that expanded (A), compressed (B), or did not change (C) their frequency range under modulation. □, and ○ indicate nonsignificant synchronization.
enhanced or suppressed. With modulation, neurons that showed an enhanced response tended to have their frequency range expanded, while suppressed neurons tended to have their frequency range compressed. For enhancers, the overall expansion was 65% and for suppressors, the overall compression was 57% (Fig. 9B). A smaller number of enhanced and suppressed neurons showed the opposite effect, i.e., compression and expansion, respectively. The magnitude of this compression for enhanced units and expansion for suppressed units was small: 35 and 27%, respectively.

**Effect of AM on ITD coding and ITD tuning width**

While modulation could induce large changes in firing rate, its effect on a neuron’s ITD coding and tuning width was small. To examine changes in ITD coding, we measured the peak ITD of peak-type and the trough ITD of trough-type neurons. In all cases, the measures made under modulation were subtracted from those made without modulation. Figure 10A shows the distribution of the shifts of the peak or trough ITD. The distribution is centered near zero with 68% of the peaks/troughs shifting $<100$ μs, either way (mean $= 30$ μs). The distributions for the enhanced and suppressed types were not different when considered alone.

To measure changes in ITD tuning width, we measured the width of the peak or trough at 50% between the maximum and minimum of each neuron’s composite ITD curve with and without modulation. The width estimated from the modulated composite curve was subtracted from that made from the unmodulated composite curve. Negative width changes indicate wider curves under the modulated stimulus condition. The distribution of the change in the width of the ITD tuning curve, separated for peak- and trough-type neurons, is illustrated in Fig. 10B. Similar to the change in ITD distribution (Fig. 10A), 63% of the units showed $<100$ μs width change either way. The mean width change for peak-type units was 19 μs and for the trough of the trough-type neurons –62 μs.

While individual neurons may show large changes under modulation in the ITD of the peak or trough and/or the width of their ITD functions (Fig. 10), these changes were not apparent in the population ITD functions. Figure 11 plots the population ITD function for all neurons (Fig. 11A) and for peak-type (Fig. 11B) and trough-type (Fig. 11C) units considered separately. The population function based on all neurons (i.e., peak- and trough-type, suppressed and enhanced) indicates that the overall effect of modulation is to increase discharge rate, especially in the region of the peak ITD (Fig. 11A, left). Thus one effect of modulation was to increase the signal-to-noise ratio of the ITD function. The coding of ITD remains relatively intact under modulation since the peak ITD remains unchanged (Fig. 11A, right) and the peak width is only slightly narrower (Fig. 11A, right). A Kolmogorov-Smirnov (K-S) Z test of the cumulative distributions revealed no significant

**Fig. 9.** A: distribution of the percent of enhanced and suppressed neurons subdivided into those that expanded, compressed, or did not change the frequency range of their interaural time difference sensitivity. B: mean percent change in expansion and compression for enhanced and suppressed neurons.

**Fig. 10.** Distributions of changes in ITD for peak- and trough-type neurons. A: shifts in the peak ITD of peak-type neurons (•) and in the trough ITD of trough-type neurons (○) when modulation was added. A negative shift is a shift of the modulated response in the direction of delays at or toward the contralateral ear. One outlier was removed from the distribution of trough-type neurons. B: distribution of the change in the width of the ITD function measured at 50% of the maximum discharge rate. Negative percentages indicate an increase of width with modulation. Two outliers were removed from the distribution of trough-type neurons.
viewed neuron by neuron or as a population, the overall effect of modulation on ITD coding and ITD tuning width was negligible. Finally, whether collapsing across enhanced and suppressed neurons or collapsing across peak- and trough-type neurons, modulation increased the overall discharge rate. We will discuss each of these effects of modulation in turn.

**Effects of modulation on ITD coding**

**DISCHARGE RATE.** When we divided our peak-type population into those that enhanced or suppressed their response to modulation, an unexpected property emerged: suppressed neurons had a markedly higher discharge rate to the unmodulated stimulus than enhanced neurons (Fig. 6). However, when the stimulus was modulated, this difference in discharge rate of enhanced and suppressed neurons was diminished. Because under natural conditions most sounds are amplitude modulated, this diminished difference has the net effect of making the ITD processing of these two classes of neurons more uniform.

Unlike the peak-type neurons, suppressed trough-type neurons had a higher discharge rate than enhanced neurons to the unmodulated stimulus. However, this difference was much smaller than in peak-type neurons. Thus for trough-type neurons the addition of modulation does not diminish the rate disparity between enhanced and suppressed neurons. Also unlike the peak-type neurons, the ITD function flattens under modulation for both enhanced and suppressed neurons. The functional effect of this finding is questionable since the peak ITD of trough-type neurons is almost always outside the estimated physiological range of the rabbit (approximately \( \pm 300 \mu s \)). If a plausible function for trough-type neurons is to code properties of spaciousness (Fitzpatrick et al. 2000), then this function would be unaffected because the peaks are relatively unaffected.

**FREQUENCY RANGE OF ITD SENSITIVITY.** Another feature of enhanced neurons was that they expanded their ITD frequency range, whereas suppressed neurons compressed their frequency.

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**DISCUSSION**

We have shown that almost all neurons sensitive to ITDs synchronize to the modulation envelope. AM can enhance, suppress, or have a minimal effect on the discharge rate of ITD responses. A third effect of modulation was to expand the ITD frequency range of enhanced neurons and compress this range for the suppressed neurons. We also found that, whether
range under modulation (Fig. 9). This is expected because increasing the discharge rate should have the effect of recruiting frequencies at the borders of ITD sensitivity, i.e., expansion, and conversely, decreasing the discharge rate should have the opposite effect, i.e., compression. Specifically, at the edges synchrony to the binaural beat is low and an increase in firing rate in this region would move the response toward statistically significant ITD-sensitivity (see METHODS). It is important to note that these changes are specific to the ITD frequency range that we measured and do not simply reflect an overall increase or decrease in spike rate. It was commonly observed that the neural discharge increased over a much larger range than the range of ITD sensitivity.

The function of the changes in ITD frequency range with modulation is unclear. The enhanced neurons could be viewed as generalists because they would respond to spectrally complex sounds and suppressed neurons could be viewed as specialists because they respond to more spectrally restricted sounds. Because in both cases, the neurons show a robust code for ITD, the change in frequency range is therefore related more to stimulus identification than localization. Cells specific to stimulus features and those that integrate across stimulus features are ubiquitous to all sensory systems. An unexpected result was the finding that many enhanced neurons had a compressed ITD frequency range under modulation. This often occurred when the modulation increased firing rate and created a flattened ITD function. On the other hand, the explanation is unclear for the small number of suppressed neurons that expanded their ITD frequency range under modulation. The magnitude of this expansion was small (27%) and may reflect, in part, physiological variability.

Effects of modulation on ITD coding and ITD tuning width

Some neurons showed large shifts in peak/trough ITD and peak/trough width with modulation (Fig. 10). These changes may, in part, be due to difficulties in estimating these parameters when the response rate is low or the response is highly synchronized to the envelope. For example, in enhanced neurons, the discharge rate could be low to the unmodulated signal. In such cases, estimating the peak/trough ITD and width may be less reliable than for the condition that elicits the higher rate. This explanation is consistent with the observation that the shifts could occur in either direction and the distribution did not change if the data were parcelled into enhanced and suppressed neurons. Furthermore, the mean changes in peak/trough ITD and width were small for both peak- and trough-type neurons.

When the ITD functions were viewed as a population, for peak-type, trough-type or both types together, the changes in peak width (peak-type, peak- and trough-type) or trough width (trough-type) were negligible under modulation (Fig. 11). Similarly, for peak ITD, the changes under modulation were also negligible for peak-type and both types combined. However, the trough ITD changed by ~100 µs under modulation, but because of the smaller proportion of trough-type units, this change had little if any effect when all neurons were considered.

In summary, the negligible changes in peak/trough ITD and width suggest that modulation, which commonly occurs in natural sounds, does not change the ITD coding of IC neurons. In a sense, this is expected because we did not vary the ITD of the envelope. It could be argued that in natural sounds, the fine structure as well as the envelope is delayed. However, it has been shown with human listeners that delaying the envelope in low-frequency sounds has negligible effects on ITD discrimination or lateralization (Hennig 1980; Schiano et al. 1986). However, it should be noted that although ITD coding remains relatively unchanged, the neuron is able to simultaneously encode the temporal features of the modulation.

Effects of modulation on ITD coding: population output

Whether we consider all neurons or focus on peak-type neurons (which are usually tuned to small ITDs within the physiological range), the peak discharge rate is increased under modulation. This occurred despite the fact that, in our experiments, the modulated stimulus contained less energy. Although the functional import of this increased response is not clear, one possibility is that it may be related to the percept created by ITDs. In tones, ITDs create a diffuse intracranial image. When the bandwidth of the sound is increased, the image becomes more compact. This compact image is so salient compared with that produced by a tone that this stimulus is commonly used in psychophysical studies exploring the lateralization of ITDs (Bernstein and Trahiotis 1985). Even though the image is more compact, for a given ITD, both tones and narrowband noise signals are lateralized to the same intracranial position. This is consistent with our observation that the ITD functions to unmodulated and modulated signals were similar in shape and peak position.

The present paper has described the responses of IC neurons to unmodulated and modulated signals and possible functional significance. In the companion paper (D’Angelo et al. 2003), we will investigate the neural mechanisms that underlie the creation of enhanced and suppressed responses under the modulated condition.

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DISCLOSURES

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