Temporal Features of Spectral Integration in the Inferior Colliculus: Effects of Stimulus Duration and Rise Time

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Submitted 9 December 2008; accepted in final form 24 April 2009

Gans D, Sheykholeslami K, Peterson DC, Wenstrup J. Temporal features of spectral integration in the inferior colliculus: effects of stimulus duration and rise time. J Neurophysiol 102: 167–180, 2009. First published April 29, 2009; doi:10.1152/jn.91300.2008. This report examines temporal features of facilitation and suppression that underlie spectrally integrative responses to complex vocal signals. Auditory responses were recorded from 160 neurons in the inferior colliculus (IC) of awake mustached bats. Sixty-two neurons showed combination-sensitive facilitation: responses to best frequency (BF) signals were facilitated by well-timed signals at least an octave lower in frequency, in the range 16–31 kHz. Temporal features and strength of facilitation were generally unaffected by changes in duration of facilitating signals from 4 to 31 ms. Changes in stimulus rise time from 0.5 to 5.0 ms had little effect on facilitatory strength. These results suggest that low frequency facilitating inputs to high BF neurons have phasic-on temporal patterns and are responsive to stimulus rise times over the tested range. We also recorded from 98 neurons showing low-frequency (11–32 kHz) suppression of higher BF responses. Effects of changing duration were related to the frequency of suppressive signals. Signals <23 kHz usually evoked suppression sustained throughout signal duration. This and other features of such suppression are consistent with a cochlear origin that results in masking of responses to higher, near-BF signal frequencies. Signals in the 23- to 30-kHz range—frequencies in the first sonar harmonic—generally evoked phasic suppression of BF responses. This may result from neural inhibitory interactions within and below IC. In many neurons, we observed two or more forms of the spectral interactions described here. Thus IC neurons display temporally and spectrally complex responses to sound that result from multiple spectral interactions at different levels of the ascending auditory pathway.

INTRODUCTION

For many auditory neurons, responses to complex sounds depend on neural mechanisms activated by frequencies well outside a neuron’s excitatory receptive field or inhibitory sidebands. Integration of spectrally distinct acoustic signal components occurs in neurons from the auditory nerve to auditory cortex and has been shown by disparate methods including neuroethological approaches (Fuizessery and Feng 1983; Margoliash and Fortune 1992; Suga et al. 1978), response area assessments (Shofner and Young 1985; Young and Brownell 1976), two-tone paradigms (Mittmann and Wenstrup 1995; Sachs and Kiang 1968; Sutter et al. 1999), spectrotemporal receptive fields (deCharms et al. 1998; Theunissen et al. 2000), and intracellular recording (Machens et al. 2004; Voytenko and Galazyuk 2007; Xie et al. 2007). Many of these approaches have shown that the temporal features of the distant spectral elements, and the temporal features of the response to those elements, can significantly affect the response to complex signals (Olsen and Suga 1991; O’Neill and Suga 1979; Sen et al. 2001; Voytenko and Galazyuk 2007). In this study, we compare the temporal features of stimuli and their evoked responses as a tool to examine the inputs and mechanisms underlying spectral integration.

This study examines forms of spectrally integrative responses prevalent in the inferior colliculus (IC) of the mustached bat. Combination-sensitive facilitation, underlying selective responses to the bat’s social and sonar vocal signals (Esser et al. 1997; Ohlemiller et al. 1996; O’Neill and Suga 1979; Suga et al. 1978, 1979), is an enhanced response that occurs when two spectrally distinct signals are combined in an appropriate temporal relationship. Such facilitation does not occur in auditory brain stem structures (Marsh et al. 2006; Portfors and Wenstrup 2001), but instead seems to originate in IC (Mittmann and Wenstrup 1995; Nataraj and Wenstrup 2005; Wenstrup and Leroy 2001; Wenstrup et al. 1999). Recent work has shown that response facilitation in IC neurons is independent of glutamatergic inputs but requires both low- and high-frequency–tuned glycinergic inputs (Sanchez et al. 2008). A likely source of these glycinergic inputs is the ventral nucleus of the lateral lemniscus (VNLL) (Wenstrup et al. 1999; Winer et al. 1995). Across species, many VNLL neurons have onsets of different temporal response patterns (Batra and Fitzpatrick 1999; Covey and Casseday 1991; Metzner and Radite-Schuller 1987; Portfors and Wenstrup 2001; Zhang and Kelly 2006). The correspondence between glycinergic VNLL neurons and onset response properties is particularly strong in bats (Covey and Casseday 1991; Portfors and Wenstrup 2001; Vater et al. 1997; Winer et al. 1995). If VNLL onset neurons contribute to combination-sensitive facilitation in the mustached bat, we predicted that facilitatory interactions should remain unaltered by changes in the duration of low-frequency facilitatory signals.

Many IC neurons show suppressive effects of stimuli at frequencies well below their best/characteristic frequency (Mittmann and Wenstrup 1995; Nataraj and Wenstrup 2005, 2006; O’Neill 1985; Portfors and Felix 2005; Portfors and Wenstrup 1999). Previous studies in the mustached bat suggest two different forms of suppressive spectral interactions. In one form, low-frequency suppression has broad tuning generally in the 10- to 22-kHz frequency range, has thresholds generally exceeding 60 dB SPL, and is sometimes accompanied by an excitatory response to the low-frequency signal (Marsh et al. 2006; Nataraj and Wenstrup 2006). These features are consistent with two-tone suppressive interactions and accompanying
low-frequency excitatory responses observed in high best (characteristic) frequency (BF) auditory nerve fibers (Arthur et al. 1971; Delgutte 1990b; Kiang and Moxon 1974; Sachs and Kiang 1968), features that originate in the cochlea (Ruggero et al. 1992; Temchin et al. 1997). Because two-tone suppression in the auditory nerve lasts for the duration of a suppressive signal (Arthur et al. 1971), suppression in IC that consistently shows a similar time course may suggest a cochlear origin.

A second form of low-frequency suppression in IC neurons (Mittmann and Wenstrup 1995; O’Neill 1985; Portfors and Wenstrup 1999) seems to depend on inhibitory neural interactions that may arise either within or below the IC (Nataraj and Wenstrup 2006; Peterson et al. 2008; Portfors and Wenstrup 2001). These suppressive neural interactions may depend on inputs from onset neurons in VNLL (Wenstrup et al. 1999), and therefore may display duration-insensitive timing. We hypothesize that sensitivity of IC neurons to the duration of low-frequency suppressive sounds will show differences in the underlying suppressive mechanisms.

To address these issues, we recorded extracellular responses from single IC neurons showing spectral facilitatory and/or suppressive interactions. We varied the duration of the interacting low-frequency signal, but also its rise-fall time because this may show additional temporal sensitivity. The results indicate that facilitatory interactions were relatively unaffected by changes in duration, suggesting that facilitatory inputs to IC neurons have onset temporal patterns. An increase in rise time altered the temporal features of interactions but did not otherwise affect it. For suppressive interactions, the results suggest two types of low-frequency interaction that, in general, differ both in frequency tuning and duration sensitivity.

METHODS

We examined auditory responses in the IC of 11 wild-caught awake mustached bats (Pteronotus parnellii), captured in Trinidad and Tobago. All procedures were approved by the Northeastern Ohio Universities College of Medicine Animal Care and Use Committee and administered following the National Institutes of Health guidelines for the care and use of laboratory animals.

Surgery

Before surgery, each bat was medicated with the sedative butorphanol (5 mg/kg, Fort Dodge Animal Health, Fort Dodge, IA) and atropine (0.06 mg/kg, Phoenix Scientific, St Joseph, MO) to prevent bronchial secretions. The bats were then anesthetized with isoflurane (1.5–2.0%, Abbott Laboratories, North Chicago, IL) and placed in a stereotaxic holder. Depilatory lotion was used to remove hair over the skull, and the skin on the head was disinfected with betadine. During surgery, a midline incision was made in the skin, and the underlying muscles were reflected laterally to expose the dorsal surface of the skull. A metal pin was cemented onto the rostral portion of the skull to secure the head during physiological experiments. Using surface and stereotaxic coordinates, a small hole (<0.5 mm diam) was opened to expose the IC. A second, smaller hole was made near the lateral surface overlying the cerebral cortex, and a tungsten wire was cemented to serve as ground for electrophysiological recordings. After surgery, Lidocaine (a local anesthetic) was applied to the surgical areas and the bat was returned to the holding cage. The bat was allowed to recover for 2–3 days before physiological experiments were initiated.

Acoustic stimulation

Single and multiple tone bursts of variable duration (4–31 ms) and rise/fall times (0.5 or 5.0 ms) were synthesized on a computer, downloaded to a digital signal processor (Microstar DAP 5216A), and converted to analog signals at a sampling rate of 400 kHz. The analog signals were filtered with an anti-aliasing filter (model FT6-2, Tucker-Davis Technologies), attenuated (model P5, Tucker-Davis Technologies), and amplified (model HCA-800II, Parasound). The signals were connected to a loudspeaker (Infinity EMIT-B tweeter, Harmon International Industries, Woodbury, NY) that was placed 10 cm from the contralateral ear and 25° into the sound field contralateral to the recorded IC. Sound repetition rate was constant at 4/s, with a 100- or 150-ms peristimulus record duration for neural responses. The performance of the speakers was tested with a calibrated microphone (model 4135, Bruel and Kjaer). There was a smooth decrease of ~3 dB per 10 kHz from 10 to 120 kHz. Distortion components were not detectable ~55 dB below the signal level.

Physiological recording

For physiological experiments, bats were placed in a stereotaxic apparatus within a heated single-walled acoustic chamber. To minimize distress, bats were lightly sedated with butorphanol (2.5 mg/kg, IP). Recording sessions never exceeded 6 h in a single day.

Data were obtained only from well-isolated single neurons located within the IC. Glass micropipettes with tips ranging from <1 to 6 μm were filled with physiological saline or 1 M NaCl and advanced dorso-ventrally into the IC with a hydraulic micropositioner (model 650, David Kopf Instruments). Extracellular action potentials were amplified, band-pass filtered (600–6,000 Hz), and connected to a spike signal enhancer (model 40-46-1, Frederick Haer and Co.) before being digitized at a sampling rate of 40 kHz (DAP 5216A, Microstar).

Consistent with the neuroethological literature, BF refers to the frequency at which the lowest sound level elicited stimulus-locked action potentials. Both BF and minimum threshold (MT) were obtained with single tone burst stimuli. BF was measured with a resolution 1/10th of a kilohertz but expressed here in kilohertz. MT was measured to the nearest decibel. Because many IC neurons respond best to combinations of distinct spectral elements in sounds (combination sensitivity), two-tone stimulus paradigms were used to evaluate the underlying spectral interactions of these units. The high-frequency tone was set at the unit’s BF, 10–15 dB above its threshold. The second tone (a lower-frequency tone) was varied over a range of frequencies, sound levels, and delays relative to the BF signal. Because the potential parameter space is very large, we searched manually for frequencies, levels, and delays that were known to activate low-frequency effects on the responses to higher-frequency signals at the BF of IC neurons (Leroy and Wenstrup 2000; Mittmann and Wenstrup 1995; Nataraj and Wenstrup 2005, 2006; Portfors and Wenstrup 1999, 2002). Once low-frequency facilitatory or suppressive effects on BF responses were observed, the best interacting frequency was identified to a resolution of 0.5–2.0 kHz. This resolution was sufficient to identify frequency-specific effects of these low-frequency interactions (Fig. 1A). Interactions <23 kHz were particularly broadly tuned. Over the course of these and contemporaneous studies (Marsh et al. 2006; Nataraj and Wenstrup 2006), we more closely attended to responses in the 10–22 kHz range. As a result, it is likely that the occurrence of suppressive responses within this band is more common than described here. Once an interaction was detected, the sound level of the low-frequency signal was raised 5–10 dB for quantitative tests described below.

In units that showed low-frequency influences, quantitative measures of delay-sensitive facilitation and/or suppression were obtained and compared with the single-tone responses. For these tests, the temporal features of the BF tone remained constant, whereas the duration and rise/fall time of the low-frequency tone was varied across
The study examined temporal features of spectral interactions in neurons recorded from the mustached bat’s IC. BFs ranged from 39 to 109 kHz, with thresholds ranging from 8 to 62 dB SPL. Among these neurons, sounds at significantly lower frequencies either enhanced or suppressed the response to the BF signal. Of 160 neurons from which we obtained sufficient quantitative data, 98 (61%) displayed suppression of
BF responses by low-frequency sounds, whereas the remainder (n = 62, 39%) displayed low frequency–evoked facilitation that was often accompanied by suppression. We also report observations from 17 neurons showing two or more forms of low-frequency interaction.

Low-frequency facilitating interactions were tuned in the range of 16–31 kHz. With the BF sound presented 10–15 dB above threshold, the sound level required for low-frequency facilitation (42–80 dB SPL) was on average 26.1 ± 13.4 dB higher than for the BF excitatory response (P < 0.001, paired t-test).

Low-frequency suppressive interactions had BF of suppression in the range 11–32 kHz, with thresholds in the range of 22–75 dB SPL (for BF sounds 10–15 dB above thresholds). As described previously (Nataraj and Wenstrup 2006), there was a marked difference in threshold for suppression as a function of the best suppressive frequency. Thus suppression tuned <23 kHz had on average 13.8 dB higher thresholds than suppression tuned ≥23 kHz (P < 0.001, unpaired t-test). As a result, there was greater disparity in sound levels used to evoke BF excitation and low-frequency suppression when the suppression was tuned <23 kHz. Thus sound levels required for suppression tuned <23 kHz were on average 47.6 ± 13.1 dB higher than for the BF excitatory response (P < 0.001, paired t-test). In contrast, sound levels required for suppression tuned ≥23 kHz were on average 26.5 ± 16.7 dB higher than for the BF excitatory response (P < 0.001, paired t-test).

Temporal features of facilitating interactions

The main objective of these experiments was to characterize the temporal properties of low frequency–evoked facilitation and suppression. We therefore examined how variations in temporal features of low-frequency sounds (duration and rise–fall times) altered the delay function of two-tone interactions— the response as a function of the relative timing of low-frequency and higher-frequency (BF) signals.

We first consider potential changes in the delay function that may result if changes in low-frequency duration alter the timing of evoked facilitation or suppression (Fig. 2A). With short-duration signals, the combination response shows a sharp peak (or trough, for suppressive interactions) in the delay function because the low and high frequency–evoked facilitatory influences are temporally restricted (Fig. 2A, top row). When the duration of the low-frequency facilitating signal is increased, one possible effect is that the low-frequency response is unaffected. This would occur if the low-frequency response is insensitive to low-frequency duration but is locked to the onset of the low-frequency sound. Under these conditions, there would be no change in the features of the delay function (Fig. 2A, Onset Facilitation). A second result would occur if the low-frequency facilitating response was locked to the offset of the low-frequency signal. The timing but not the duration of the low-frequency facilitatory input would change. The result is that the delay function would retain the same shape but the CS_{START}, peak and CS_{END} measures would shift to later delays (Fig. 2A, Offset Facilitation). A third possibility is that the facilitating influence extends throughout the duration of the low-frequency signal. As a result, the range of delays over which facilitation would occur is broader, and the CS_{END} measure would shift to longer delays (Fig. 2A, Sustained Facilitation). Although effects of low-frequency duration are shown for facilitatory interactions, suppressive interactions could show similar effects.

The rise time of low-frequency signals may also influence the delay function of combination-sensitive interactions. Figure 2B (top row) shows a facilitatory interaction evoked by signals with fast rise times (0.5 ms). When the rise time of the low-frequency signal is increased, the latency of the low-frequency facilitating input would be increased because the sound level reaches threshold later (Fig. 2B, Onset Facilitation). As a result, CS_{START}, peak, and CS_{END} measures would shift to later delays. Corresponding changes would be expected if low-frequency facilitation were of an offset or sustained type. Another possible result is that neurons that provide low-frequency facilitating (or inhibitory) inputs to combination-sensitive IC neurons may be unresponsive to longer rise times (Fig. 2B, No Facilitation). The same may also occur for low-frequency sound durations (data not shown).

Temporal features of facilitating interactions

Among 62 facilitated neurons, we found little evidence that changes in the duration of low-frequency facilitating signals caused systematic changes in the facilitation delay function. However, low-frequency rise time generally altered features of the delay curve. For the neuron in Fig. 3, delay functions changed little as the duration of the low-frequency facilitating signal was increased from 4 to 13 to 31 ms. For all three functions, the delay-tuned peak remained at 8 ms, and the initial rise (CS_{START}) and fall (CS_{END}) of the delay function varied by <1.0 ms. These results strongly suggest that the facilitating effect of the low-frequency signal is phasic and locked to the onset of the low-frequency signal. The delay function was more strongly affected by a change in the rise time of the low-frequency signal, from 0.5 to 5 ms. The longer rise time shifted the rising and falling edges of the delay function by 3.5 and 3.9 ms, respectively, and increased the best delay by 2 ms. Thus in this neuron, features of the facilitation delay function were shaped by stimulus rise time but the facilitation occurred over a range of rise times.

Delay functions in two other neurons (Fig. 4) show additional features of changing low-frequency duration: 1) low-frequency sound duration altered delay functions somewhat, but these effects were limited, and 2) the limited effect of duration occurred in neurons for which the width of the delay function was quite different. For the neuron in Fig. 4A, the rising phase (CS_{START}) of the delay function was unaffected by low-frequency duration. The falling phase (CS_{END}) increased by 3.9 ms as duration was changed from 4 to 13 ms but increased only slightly (1.3 ms) as duration changed from 13 to 31 ms. The width of the delay function ranged from 8.9 to 15.0 to 15.3 ms for low-frequency durations from 4 to 13 to 31 ms, respectively. These functions were among the widest observed in the study. In contrast, the neuron in Fig. 4B, had a particularly narrow facilitatory delay function (3.3–5.7 ms) that showed only very minor changes with increasing sound duration. Thus the rising phase of the delay function (CS_{START}) was unaffected by low-frequency duration. The falling phase (CS_{END}) increased by 2.4 ms as duration changed from 4 to 13.
ms, but did not change further as duration changed from 13 to 31 ms. For both neurons, the duration of the facilitatory effect increased slightly as low-frequency sound duration increased from 4 to 13 ms, but further increases in low-frequency duration did not further lengthen the facilitatory effect. On the assumption that the width of the delay function is a reflection of the duration of the underlying sound-evoked facilitatory influence, these results suggest that the facilitatory effect is phasic and locked to the onset of the low-frequency stimulus (Fig. 2, Onset Facilitation). The lack of change in the rising phase of the delay curve (CSSTART) is not consistent with offset facilitation, whereas the lack of substantial change in the falling phase of the delay function (CSEND) is not consistent with sustained facilitation (Fig. 2).

To study how changes in low-frequency duration affected delay functions of the population of facilitated neurons, we compared the measures of facilitation delay functions for different low-frequency durations. In Fig. 5, A and B, we plotted the timing of the leading edge of the delay function (CSSTART) for 4- versus 13-ms durations (Fig. 5A) and for 4- versus 31-ms durations (Fig. 5B). As described in Fig. 2A, onset- and sustained-type facilitatory interactions show no change in CSSTART at different low-frequency durations, corresponding to data scatter along the solid diagonal line ($y = x$) in Fig. 5, A and B. Offset-type facilitatory interactions would show a shift in CSSTART by 9 ms for low-frequency duration of 13 ms and by 27 ms for 31-ms duration. If the facilitation is offset-type, data should scatter along the dashed diagonal lines in Fig. 5, A and B ($y = x + 9$ and $y = x + 27$, respectively). This did not occur. Although the rising phase of delay curves did not generally remain identical as the low-frequency duration was changed, there was no evidence of a systematic shift in the CSSTART measure that is consistent with offset-type facilitation.

FIG. 2. Rationale and predictions for tests that evaluate the effects of changes in low-frequency duration (A) and rise time (B). On the left are schematic postsynaptic potentials and spikes associated with responses to low-frequency (L, filled hexagons) and high-frequency (H, unfilled hexagons) stimuli. On the right are delay curves showing spike discharge as a function of relative timing of low-frequency and BF signals. The grayed, dashed line represents the control delay function obtained under conditions of 4-ms duration, 0.5-ms rise/fall (r/f) for the low-frequency signal.
were statistically significant increases in CS$_{START}$ (mean change: 5.7 ms, range: 0.4–15.3 ms). Slower stimulus rise and fall times caused alterations in delay functions; a change in low-frequency rise time shifted the delay function to the right.

In Fig. 5, C and D, we compared the timing of the trailing edge of the delay function (CS$_{END}$). As described in Fig. 2A, onset-type facilitation would result in no change in CS$_{END}$ corresponding to data scatter along the solid diagonal lines in Fig. 5, C and D. In contrast, both sustained- and offset-type facilitation would result in an increase in CS$_{END}$ corresponding to data scatter along the dashed diagonal lines in Fig. 5, C and D. Again, there is variability in this measure across duration, but no consistent shift in the CS$_{END}$ of delay functions with longer duration signals. For the population, these results strongly support a hypothesis that low-frequency facilitating responses are locked to stimulus onset and are mostly insensitive to stimulus duration. With low-frequency signals of 31-ms duration, the duration of facilitation averaged 5.3 ± 3.7 ms (range: 0.4–15.3 ms).

Across the sample of facilitated neurons, the duration of the low-frequency signal had no significant effect on the strength of the facilitatory interaction (Fig. 6, A and B). Only a small number of neurons gained ($n = 2$) or lost ($n = 6$) facilitation with an increase in low-frequency duration.

Slower stimulus rise and fall times caused alterations in temporal features of facilitation but not, generally, in the strength of facilitation. Across the sample of facilitated neurons studied for effects of rise and fall times ($n = 58$), there were statistically significant increases in CS$_{START}$ (mean change: 2.7 ± 4.3 ms, $P < 0.001$, paired $t$-test) and CS$_{END}$ (mean change: 5.7 ± 5.4 ms, $P < 0.001$, paired $t$-test). These results are consistent with the prediction in Fig. 2B (Onset Facilitation) that a slower rise/fall time shifts the delay curve to the right. However, the longer rise/fall times had little effect on the strength of facilitation of most neurons (Fig. 6C). Across the sample of facilitated neurons tested, we observed no significant change in the strength of facilitation as the rise/fall time increased from 0.5 to 5.0 ms. A small number of neurons showed facilitation only with longer rise/fall times ($n = 2$). A somewhat larger group ($n = 7$) showed facilitation only with the shorter rise/fall time of 0.5 ms.

**Temporal features of suppressive interactions**

Among the 98 neurons showing suppressive low-frequency interactions, changes in low-frequency duration had a range of effects on delay sensitivity functions. The two neurons in Fig. 7 showed delay functions for which large changes in low-frequency duration had little effect on delay tuning. Low-frequency suppression appeared to be onset related (no change in CS$_{START}$) and not significantly increased at longer durations (little or no change in CS$_{END}$). These duration-insensitive suppression functions occur both in neurons with no low-frequency excitatory response (Fig. 7A) and in neurons with an excitatory low-frequency response that varies with duration (Fig. 7B). For other neurons, low-frequency duration significantly altered delay functions (Fig. 8). These neurons showed no change in the onset of the suppression (no change in CS$_{START}$) as the low-frequency duration increased, but displayed a significant increase in CS$_{END}$. As a result, there was an increase in the range of delays over which low-frequency suppression occurred, nearly matching the increase in stimulus duration. This suggests that, in these neurons, low-frequency suppression was locked to stimulus onset and sustained for the duration of the low-frequency sound. These effects were observed in neurons that displayed an absence or presence of a low-frequency excitatory response (Fig. 8, A and B, respectively). Thus the presence of the low-frequency excitatory response was unrelated to duration sensitivity of low-frequency suppression.

Previous work in mustached bats showed quantitative differences in low-frequency suppression tuned to 23–30 kHz compared with suppression tuned <23 kHz. Thus 23- to 30-kHz suppression had lower thresholds and had different temporal properties when associated with excitation (Nataraj and Wenstrup 2006). These results show an additional difference between these two bands of low-frequency suppression,
based on sensitivity to the duration of the signals. Thus low-frequency interactions tuned in the 23- to 30-kHz band showed less sensitivity to changes in duration (Fig. 7, A and B) than did interactions tuned in the lower-frequency band (Fig. 8, A and B). This is shown for the sample of neurons with low-frequency suppression in Fig. 9, in which we plot the change in $C_{SEND}$ for 4- and 31-ms signals as a function of the tuning of the low-frequency interaction. The large values for suppressive responses tuned in the 10- to 21-kHz range indicate that the suppression lengthens with signal duration. The small values of $C_{SEND}$ for suppressive responses tuned in the 22- to 31-kHz range indicate that the duration of suppression did not generally change with increases in signal duration. Although a dividing frequency may be somewhat arbitrary and there are some individual exceptions, this analysis supports previous distinctions between suppression in the 10- to 22- and 23- to 30-kHz bands. Subsequent analyses of duration effects are performed separately on these two frequency bands of suppression.

To study how changes in the duration and frequency tuning of low-frequency suppressing sounds affected delay functions, we compared the timing of the onset ($CS_{START}$) and offset ($CS_{END}$) of suppression for different durations of low-frequency sounds. This population analysis supports conclusions that low-frequency suppression tuned <23 kHz is different from low-frequency suppression tuned in the range of 23–30 kHz and that this difference is mainly in the duration of the suppression. Thus there is no difference in timing of the onset of low-frequency suppression (similar $CS_{START}$) that relates to frequency tuning of suppression or to the duration of the low-frequency signal (Fig. 10, A and B). The principal difference, instead, relates to the timing of the offset of suppression ($CS_{END}$). For neurons with suppression tuned <23 kHz, the suppression significantly increased with longer-duration stimuli (Fig. 10, C and D, • scattered along dashed diagonal line). In contrast, suppression tuned in the range of 23–30 kHz changed little, even for longer-duration, 31-ms signals (Fig. 10, C and D, ○ scattered more along solid diagonal line).

The tuning of low-frequency suppression was also associated with different relationships between signal duration and the strength of suppression (Fig. 11, A and B). For suppression tuned <23 kHz, the strength increased significantly as signal duration was increased beyond 4 ms. This was not the case for suppression tuned in the 23- to 30-kHz range.

As for facilitatory interactions, the timing of low-frequency suppression was altered by changes in the rate of rise and fall of tonal stimuli, as predicted in Fig. 2B. Thus slower rise times shifted features of suppressive delay functions (both $CS_{START}$ and $CS_{END}$) to significantly longer delays ($P < 0.01$, multiple
paired $t$-tests, data not shown). This effect was independent of the frequency tuning of suppression. The strength of suppression was not significantly altered by the rate of rise of low-frequency stimuli (Fig. 11C), and this was also independent of the frequency tuning of suppression.

**Multiple spectral interactions in IC neurons**

As this study progressed, it became clear that many neurons showed more than one of the types of spectral interactions described above. When a subset of neurons ($n = 17$) was systematically studied for multituned effects, 41% ($n = 7$) were found to exhibit sustained suppression tuned to frequencies $< 23$ kHz and phasic suppression ($n = 5$) or facilitation ($n = 3$) $\geq 23$ kHz. The neuron in Fig. 12 showed all three forms of low-frequency interaction described in this study: facilitation and suppression tuned near 27 kHz (Fig. 12A) and suppression tuned near 20 kHz (Fig. 12B). We describe this neuron’s response below to clarify the complex features of its low-frequency interactions.

The response to brief (13 ms) BF tones (58 kHz) was facilitated by 27-kHz tone bursts at some delays and was suppressed by 27-kHz tones at other delays (Fig. 12A). BF tones presented individually evoked aphasic discharge, whereas...
brief (4 ms) tones at 27 kHz evoked slightly less spike discharge. Particularly noteworthy was the long latency of the BF excitatory response (26 ms) and the very long latency of the 27-kHz response (42 ms). Because the latencies of the excitatory responses to the individual tones are closely correlated with the latency of facilitatory influences (Portfors and Wenstrup 1999; Sanchez et al. 2008), the latency difference predicts a best delay of 16 ms for the strong facilitatory response. This is close to the observed best delay of 18 ms. This facilitatory interaction was virtually identical with low-frequency duration of 4 and 31 ms, both in terms of the delay function and temporal response patterns (Fig. 12A, peristimulus time histograms). There was strong suppression of the BF response by the 27-kHz signal over a range of delays from −10 (BF signal leading) to +4 ms. This suppression is likely related to the very long latency of the BF excitatory response, and we hypothesize that it depends on a short latency but long-lasting suppression evoked by the 27-kHz signal. The 27-kHz suppression seems to be independent of signal duration, because the delay function and temporal response patterns are nearly identical under the 4- and 31-ms conditions.

After completing two-tone tests with BF and 27-kHz signals, we examined effects of 20-kHz tones on BF (Fig. 12B). At this time, the response to the BF signal doubled compared with responses shown in Fig. 12A, but we confirmed that other response properties including facilitation and response latency were the same. The response to the 20-kHz signal was duration sensitive: weak at 4-ms duration and strong at 31-ms duration. When the BF and 20-kHz signals were presented together, the 20-kHz signal suppressed BF responses over a range of negative delays (BF signal leading), the result of the very long latency of the BF response. In contrast to suppression by 27-kHz responses, the 20-kHz suppression was duration sensitive, increasing in strength and duration as the signal duration increased.

Because we only examined the co-occurrence of such responses later in this study, the exact proportions of neurons with suppression <23 kHz and either facilitation or suppression in the 23- to 30-kHz range is unclear. It is our sense, however, that suppressive effects of signals <23 kHz are quite common, occurring in most if not all high-frequency tuned neurons of the IC.

**DISCUSSION**

This study examined temporal features of spectral interactions that contribute to neuronal responsiveness to complex sounds in the IC. We sought to characterize the inputs and mechanisms underlying these interactions in IC. Major observations are that facilitatory interactions are locked to the onset of a low-frequency facilitating signal and are largely insensitive to its duration. These interactions may depend on auditory brain stem inputs that show onset-type temporal response patterns. Suppressive interactions are diverse in their response to changes in duration, suggesting that multiple mechanisms or inputs underlie such interactions. The temporal sensitivity of both facilitatory and suppressive interactions suggests a variety
of effects that can contribute to the response to complex sounds. For many neurons, we observed more than one type of interaction, resulting in even more elaborate responses to complex sounds.

Changes in the rate of rise and fall of lower-frequency tone bursts altered the time course of facilitatory and suppressive interactions by shifting delay functions to larger values. This result is expected, arising from a delay in the time required for the low-frequency sound to reach threshold. This shows that our measurements were sufficiently sensitive to detect small changes in the temporal features of two-tone interactions. Other features of interactions such as strength were generally unaffected by changes in rise and fall times, suggesting that the mechanisms that create low-frequency facilitation or suppression operate well in the range used in this study.

Temporal properties of facilitation in combination-sensitive neurons

Facilitation, referring to a nonlinear enhancement of a neuron’s response to an input in the presence of a second input, occurs in many neural systems. In sensory systems, it creates selectivity to complex stimuli including the direction of motion of a visual object (Clifford and Ibbotson 2003), the electric field of weakly electric fish (Carlson and Kawasaki 2004), the direction of motion across whiskers (Kida et al. 2005), and combinations of odorants (Yoshida and Mori 2007) or acoustic elements in vocal signals (Suga et al. 1978). The time scales of facilitation and the underlying neuronal mechanisms vary substantially. Neurons in the medial superior olivary nucleus are selective for interaural timing differences (ITDs) in the submillisecond range, and this selectivity seems to be based in part on facilitation (Goldberg and Brown 1969; Pecka et al. 2008; Yin and Chan 1990). This time scale of facilitation depends on several factors, including precisely timed inputs from each cochlear nucleus (Joris et al. 1994), as well as fast excitatory postsynaptic potentials and ionic currents that limit temporal integration (Kuba et al. 2002; Scott et al. 2005; Svirskis et al. 2004). At a longer time scale, neurons in the superior colliculus are facilitated by multimodal sensory inputs that are distributed over tens and hundreds of milliseconds; these facilitatory inputs depend in part on the slower action of NMDA receptors (Binns 1999). The nervous system thus uses an array of mechanisms to create facilitation on the “desired” time scale.

In the mustached bat, facilitatory spectral interactions arise through the integration of low and high frequency–tuned inputs
by single neurons in the IC. The facilitating inputs, which are glycinergic and may depend on postinhibitory rebound (Nataraj and Wenstrup 2005; Sanchez et al. 2008; Wenstrup and Leroy 2001), seem to interact independent of the glutamatergic inputs that establish the neuron’s response to sounds near its BF (Peterson et al. 2008; Sanchez et al. 2008). This study showed that the facilitating effect of low-frequency signals is locked to stimulus onset, is not activated by stimulus offset,
and is generally insensitive to increases in signal duration beyond 4–13 ms. The results further suggest that the low-frequency facilitating effect is temporally restricted, lasting ~5 ms on average. Our interpretation is that the glycinergic inputs, or at least the low-frequency glycinergic input, have a phasic-on response to sound. We further speculate that the phasic-on spikes of the glycinergic input neurons then activate a hyperpolarization with a duration dictated by postsynaptic passive and active mechanisms and not by stimulus duration. This is followed by a rebound depolarization with timing also dependent on passive and active postsynaptic membrane properties, but independent of stimulus duration.

The mostly likely sources of facilitating glycinergic inputs are neurons in the ventral (VNLL) and intermediate nuclei (INLL) of the lateral lemniscus. Glycinergic neurons occur in each of these nuclei (Aoki et al. 1988; Saint Marie et al. 1997; Vater et al. 1997; Winer et al. 1995), and nearly all small spherical cells in the “columnar” subdivisions of echolocating animals are glycinergic (Vater et al. 1997; Winer et al. 1995). Moreover, VNLL and INLL have the largest numbers of brain stem neurons that project to facilitative combination-sensitive regions of the IC, and some of the projecting VNLL and INLL neurons occur in putative low-frequency regions (Wenstrup et al. 1999; Yavuzoglu and Wenstrup 2008). Neurons with onset-type temporal response patterns, which seem to characterize the facilitating inputs, are common in INLL and VNLL, especially in the columnar subdivision of VNLL (Covey and Casseday 1991; Haplea et al. 1994; Metzner and Radke-Schuller 1987; Portfors and Wenstrup 2001). Although other brain stem nuclei may contribute onset-type facilitating glycinergic input to IC (e.g., medial superior olive; Grothe 1994), there is no indication that their low-frequency regions project to the high-frequency IC regions containing combination-sensitive neurons (Wenstrup et al. 1999; Yavuzoglu and Wenstrup 2008). We therefore propose that inputs from onset-type neurons in VNLL or INLL are crucial for establishing the facilitated combination-sensitive response in IC neurons.

These facilitating glycinergic inputs create sensitivity to combinations of neural elements that are separated by delays in the range of 0–30 ms. The degree of selectivity, on average ~5 ms, is established by the phasic nature of the low-frequency, and probably high-frequency, facilitating glycinergic input, as well as the temporal restriction on the depolarizing influence of the phasic glycinergic inputs. The temporal properties of interactions described here are thought to create selectivity to pulse-echo delay and other analyses in the mustached bat (Olsen and Suga 1991; O’Neill and Suga 1982; Portfors and Wenstrup 1999). However, both the mechanisms and their uses in acoustic analyses are more general. For example, the spatiotemporal integrative properties describe here may result, in other systems, from GABAergic inputs that are phasic and appropriately tuned in frequency. The time scales of these interactions are also appropriate for mechanisms that create selectivity to short time scale elements in vocal communication signals (Leroy and Wenstrup 2000; Olhivemiller et al. 1996; Rauschecker et al. 1995) and for analysis of phonemic elements in human speech (Suga 1996; Sussman et al. 1998).

### Temporal properties of suppression

In the mustached bat, IC neurons show two forms of low-frequency suppression of responses to BF tones, apart from lower sideband inhibition. One form is characterized by responsiveness to frequencies in the bat’s audible range <23 kHz, i.e., below the frequencies used in the first harmonic of the biosonar signal. There are several features that are more common for suppression tuned in this range: 1) it is sustained for the duration of the suppressing signal, 2) it has a relatively high threshold (typically >60 dB SPL), 3) its strength is on average greater than the other form of low-frequency suppression, becoming stronger with longer signal duration, and 4) it is often associated with excitatory responses to the low-frequency signals that precede the suppressive response (Nataraj and Wenstrup 2006; this study). This form of suppression has been observed in cochlear nuclei (Marsh et al. 2006), as well as in the IC of this species. In IC, blockade of GABAergic and/or glycinergic inhibition fails to eliminate suppression tuned <23 kHz (Nataraj and Wenstrup 2006). Thus it seems clear that its origin is below the IC.

Although we cannot rule out mechanisms operating within the lower auditory brain stem, this suppression seems most likely to be the result of cochlear mechanisms (Marsh et al. 2006; Nataraj and Wenstrup 2006). In addition to our previous characterizations of sound levels evoking this suppression, of its broad low-frequency tuning, and of its association with low-frequency excitation, this study further shows that the suppression generally lasts for the duration of the low-frequency signal. Each of these properties corresponds well to properties of two-tone suppression observed in auditory nerve fibers (Arthur et al. 1971; Delgutte 1990b; Kiang and Moxon 1974; Sachs and Kiang 1968). If this suppression is of cochlear origin, it is reasonable to expect that it would be present in the responses of all IC neurons, as it is in auditory nerve fibers (Sachs and Kiang 1968). We believe that may be the case. However, the current and previous studies have not systematically documented this because of primary focus on combination-sensitive inhibition in the 23- to 30-kHz range. Recordings of auditory nerve fibers in the mustached would help to identify the origin of these features of low-frequency responses among neurons tuned to high BFs.

Cochlear suppression and mechanisms that generate tails of excitatory tuning curves to higher frequencies may be epiphenomenal of cochlear processing, but they can have profound effects on the processing of complex sounds. Indeed, Kiang and Moxon (1974) suggested that responses to complex signals in cats are influenced by cochlear mechanisms acting within the tails of high-frequency tuning curves. Delgutte (1990a) showed that masking effects of these low-frequency signals are often associated with suppressive rather than excitatory effects. Similar observations have been reported for IC neurons in the mustached bat (Holmstrom et al. 2007; Sheykholeslami et al. 2004). The manner in which IC neurons respond to sounds in the tail/suppressive region is complex: tail excitation may be phasic or sustained, and it occludes the response to BF signals at certain delays (Nataraj and Wenstrup 2006). The suppression, in contrast, is nearly always sustained, lasting for the duration of the low-frequency signal. Depending on which of these effects predominate, significant energy in the tail region may shut down a neuron’s responses to BF signals or replace
them with excitatory responses to sounds in the tail region. In this way, high BF neurons that are tuned to the mustached bat’s sonar signal may respond in a different behavioral context, analyzing low-frequency signals with no energy near the neurons’ BFs.

The second type of suppressive low-frequency interaction is a form of combination sensitivity that seems to depend on neural inhibition. The suppression is tuned to frequencies in the range of 23–30 kHz, a limited band corresponding to the frequencies of the first harmonic of the bat’s biosonar signal but also included in many social vocalizations (Kanwal et al. 1994). This study showed that suppression tuned in this range is typically phasic and locked to the onset of the suppressive signal and that its strength is not consistently altered by changes in duration or rise time of the suppressive signal.

Inhibitory combination-sensitive interactions seem to occur both within and below the IC. Thus this form of suppression is rare in cochlear nuclei (Marsh et al. 2006) but present in lateral lemniscal nuclei (Portfors and Wenstrup 2001). In IC, blockade of GABAergic and/or glycinegic inhibition generally fails to eliminate suppression tuned in the range of 23–30 kHz (Nataraj and Wenstrup 2005, 2006). In intracellular recordings from IC neurons that show suppression by 23- to 30-kHz sounds, the majority (57%) do not show low frequency–evoked inhibitory postsynaptic potentials (IPSPs) (Peterson et al. 2008). These results support a conclusion that combination-sensitive inhibition originates in auditory brain stem nuclei below the IC. However, GABA or glycine receptor blockade significantly reduces combination-sensitive inhibition in some IC neurons (Nataraj and Wenstrup 2005, 2006), and the intracellular study observed low frequency–evoked IPSPs in 43% of combination-sensitive neurons (Peterson et al. 2008). These results suggest that low frequency–tuned brain stem neurons project to some high BF neurons in IC. Overall, these results are consistent with conclusions that 1) this neural inhibition depends on interactions within one or more lower auditory centers, 2) the results of those interactions are inherited by IC neurons via excitatory inputs, and 3) the 23- to 30-kHz inhibition is enhanced in some IC neurons by direct low-frequency inhibitory input. Combination-sensitive inhibition thus seems to be a complex response property that arises through multiple, spectrally integrative interactions in the ascending auditory pathway.

The phasic nature of this inhibition in the majority of inhibitory combination-sensitive neurons corresponds well to a presumed function in acoustic behavior: it generates a brief suppression of excitatory responses to an emitted sound with significant energy in both the 23- to 30-kHz band and a higher-frequency band near the neuron’s BF. This occurs in biosonar, where emitted sonar signals have substantial energy both at the fundamental (23–30 kHz) and higher harmonics. In echoes, the amplitude of all harmonics is reduced, but the amplitude of the fundamental may be too weak to activate this suppression. Thus low frequency–evoked inhibition is thought to suppress responses to emitted sonar signals but permits subsequent responses to returning echoes. In many cases, this low-frequency inhibition is coupled with facilitation that permits a strong response only to echoes returning at particular times, creating neurons tuned to particularly ranges of pulse-echo delay (Nataraj and Wenstrup 2005; Olsen and Suga 1991; Portfors and Wenstrup 1999).

Although this form of low-frequency suppression is particularly well documented in mustached bats in association with analysis of sonar echoes, it clearly has a broader role in analyzing complex sounds in this bat and in other species. Thus inhibition in spectrally distinct bands (vs. lateral inhibition) occurs in mustached bat IC neurons tuned to combinations outside the sonar band (Leroy and Wenstrup 2000), in mouse IC (Portfors and Felix 2005), and in cat and primate auditory cortex (Kadia and Wang 2003; Rauschecker et al. 1995; Sutter et al. 1999). These interactions contribute to analyses of a broad range of complex sounds.

ACKNOWLEDGMENTS

We thank W. E. O’Neill for discussions that initiated this study, the Auditory Neuroscience Group at NEUOCOM for discussion and comments on the manuscript, and C. Grose for preparation of the figures. We are grateful to the Wildlife Section of the Ministry of Agriculture, Land and Marine Resources of Trinidad and Tobago for permission to exports bats.

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GRANTS

This work was supported by National Institute on Deafness and Other Communication Disorders Grants R01 DC-00937 to J. J. Wenstrup and F32 DC-007786 to D. C. Peterson.

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Responses to pure tones and linear FM components of the nucleus laminaris in the chicken.


