Title: Temporal features of spectral integration in the inferior colliculus: effects of stimulus duration and rise time

Authors: Donald Gans, Kianoush Sheykholeslami, Diana Coomes Peterson, and Jeffrey Wenstrup

Affiliation: Department of Anatomy and Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272

Running head: Temporal features of spectral integration in midbrain

Pages: 45
Figures: 12

Corresponding author: Jeffrey Wenstrup, Ph.D.
Department of Anatomy and Neurobiology
Northeastern Ohio Universities College of Medicine
4209 State Route 44, PO Box 95
Rootstown, Ohio 44272
Telephone: (330) 325-6636
Fax: (330) 325-5916
jjw@neoucom.edu
ABSTRACT

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 ms to 31 ms. Changes in stimulus rise time from 0.5 ms 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 below 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 23-30 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 revealed by disparate methods including neuroethological approaches (Fuzessery 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), spectro-temporal 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 (O’Neill and Suga, 1979; Olsen and Suga, 1991; 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 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; O’Neill and Suga, 1979; Ohlemiller et al., 1996; 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 brainstem structures (Marsh et al., 2006; Portfors and Wenstrup, 2001), but instead appears 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 onset-type temporal response patterns (Batra and Fitzpatrick, 1999; Covey and Casseday, 1991; Metzner and Radtke-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 display 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 Wenstrup, 1999; Portfors and Felix, 2005). 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 10-22 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 auditory nerve fibers (Sachs and Kiang, 1968; Arthur et al., 1971; Kiang and Moxon, 1974; Delgutte, 1990b), features that originate in the cochlea (Ruggero et al., 1992; Temchin et al., 1997). Since 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) appears 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 reveal 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 since this may reveal 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.
MATERIALS AND METHODS

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

Surgery

Prior to 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 Inc., 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 in diameter) 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 to 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 then converted to analog signals at a sampling rate of 400 kHz. The analog signal(s) were filtered with an anti-aliasing filter (Tucker-Davis Technologies, model FT6-2), attenuated (Tucker-Davis Technologies, model PA5), and amplified (Parasound model HCA-800II). The signal(s) were then 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/sec, with a 100 or 150 ms peristimulus record duration for neural responses. The performance of the speakers was tested with a calibrated microphone (Brüel and Kjaer, model 4135). There was a smooth decrease of approximately 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 (0.0025 mg/kg, IP). Recording sessions never exceeded 6 hours in a single day. Data were obtained only from well-isolated single neurons located within the IC. Glass micropipettes with tips ranging from < 1 µm to 6 µm were filled with physiological saline or 1M
NaCl and advanced dorso-ventrally into the IC with a hydraulic micropositioner (David Kopf Instruments, model 650). Extracellular action potentials were amplified, bandpass filtered (600-6000 Hz), and connected to a spike signal enhancer (Fredrick Haer and Co., model 40-46-1) before being digitized at a sampling rate of 40 kHz (Microstar DAP 5216A).

Consistent with the neuroethological literature, best frequency (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 one-tenth of a kilohertz but expressed in the manuscript and figures in kilohertz. MT was measured to the nearest decibel. Since 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 below 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. 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 displayed low frequency influences, quantitative measures of delay-sensitive facilitation and/or suppression were obtained and compared to the single-tone responses. For these tests, the temporal features of the BF tone remained constant, while the duration and rise/fall time of the low frequency tone was varied across tests. Delay tests were thus obtained with the low frequency duration set at 4, 13, and 31 ms with a 0.5 ms rise/fall time as well as 13 and 31 ms duration with a 5.0 ms rise/fall time. The high frequency duration was maintained at 4 ms and only increased if the unit responded poorly to the short duration signal. We also obtained rate-level and duration tests for some neurons.

**Analysis**

We measured neural responses to the BF tone as a function of the timing of a second, lower frequency tone. Spike discharge was analyzed within a temporal window based on each neuron’s response to its BF tone. The window was set to encompass all sound-evoked spiking activity in response to the BF tone presented alone. For calculation of responses to the second tone presented alone, the same window width was used. For tests involving combinations of the two tones, the same window width was used, placed to include the neuron’s response to the BF signal.

For facilitation, neurons were considered to be combination-sensitive if their response to the combined sounds, separated by the delay providing the largest interaction effect, was at least 20% higher than the sum of the responses to the two signals presented separately. The strength
of combination-sensitive interactions was quantified by an interaction index, where the index = \( \frac{(R_C - R_{LF} - R_{BF})}{(R_C + R_{LF} + R_{BF})} \). \( R_{LF} \) is the response to the low frequency signal, \( R_{BF} \) is the response to the higher BF signal, and \( R_C \) is the response to the combination of signals. The largest positive interaction index of +1.0 corresponds to maximum facilitation. Facilitatory responses were defined as those having an index value of +0.09 or greater, corresponding to an increase of 20% above the summed response of the two signals.

Neurons were considered to display low frequency suppression if the response to the combined signal, separated by the delay providing the greatest response reduction, was at least 20% lower than the response to the BF signal presented separately. The formula used for the interaction index is \( \frac{(R_C - R_{BF})}{(R_C + R_{BF})} \). This differs from the index for facilitation by eliminating the measurement of the low frequency response presented alone. This index allowed for a more strict measure of suppression of BF by the low frequency signal, without contamination by excitatory low-frequency responses. Maximum suppression corresponds to an interaction index value of -1.0, while the 20% threshold criterion corresponds to an interaction index value of –0.11. As used here, the term “suppression” refers to any reduction in the BF response by sounds at least an octave lower in frequency, independent of the underlying mechanism.

Combination-sensitive and other two-tone interactions are characterized by spike discharge that varies with the delay between the two signals. Plots of spike discharge versus delay (= delay function) typically show a positive or negative peak (Fig. 1). Although our past studies have focused on the peak of the function (best delay) or its width (Nataraj and Wenstrup, 2005, 2006; Portfors and Wenstrup, 1999), the experimental manipulations employed here may separately alter the initial and terminal parts of the function. We estimated the onset of the interaction, termed the \( CS_{START} \), by computing and then averaging the delays at which the
function intersects lines corresponding to 25%, 50%, and 75% of the maximum facilitation or suppression (Figs. 1B,C). The offset of the facilitatory or suppressive interaction is measured similarly and is termed the CS\textsubscript{END}. The difference between CS\textsubscript{START} and CS\textsubscript{END} is the “delay width”, a measure of the duration of the spectral interaction. The delay at which the interaction is strongest is the “best delay.”

For statistical analyses, t-tests (two-tailed) were used to examine whether low frequency duration or rise time were associated with changes in the delay function. These were performed with an error (\(\alpha\)) level set to a stringent 0.01 level, to reduce chances of obtaining spurious significant results. Mean values are reported with the corresponding standard deviation (mean \(\pm\) standard deviation), except where noted in Figure 1.
RESULTS

This study examined temporal features of spectral interactions in neurons recorded from the mustached bat’s IC. Best frequencies (BFs) ranged from 39-109 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, while 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 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 best frequencies of suppression in the range 11-32 kHz, with thresholds in the range 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 mean threshold for suppression as a function of the best suppressive frequency. Thus, suppression tuned below 23 kHz had on average 13.8 dB higher thresholds than suppression tuned at or above 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 below 23 kHz. Thus, sound levels required for suppression tuned below 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 in the 23-30 kHz range was on average 26.5 ± 16.7 dB higher than for the BF excitatory response ($p < 0.001$, paired $t$-test).

**Predictions of effects of temporal manipulations**

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 displays 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 were 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\textsubscript{END} measure would shift to longer delays (Fig. 2A, “Sustained Facilitation”). Although effects of low frequency duration are illustrated 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\textsubscript{START}, peak, and CS\textsubscript{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 (not illustrated).

**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 Figure 3, delay functions changed little as the duration of the low frequency facilitating signal was increased from 4 ms to 13 ms to 31 ms. For all three functions, the delay-tuned peak remained at 8 ms, and the initial rise (CS\textsubscript{START}) and fall (CS\textsubscript{END}) of the delay function varied by
less than 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 ms 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) reveal 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 Figure 4A, the rising phase (CS\text{START}) of the delay function was unaffected by low frequency duration. The falling phase (CS\text{END}) increased by 3.9 ms as duration was changed from 4 ms 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 ms to 15.0 ms to 15.3 ms for low frequency durations from 4 to 13 ms to 31 ms, respectively. These functions were among the widest observed in the study. In contrast, the neuron in Figure 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\text{START}) was unaffected by low frequency duration. The falling phase (CS\text{END}) increased by 2.4 ms as duration changed from 4 ms to 13 ms, but did not change further as duration changed from 13 ms to 31 ms. For both neurons, the duration of the facilitatory effect increased slightly as low frequency sound duration increased from 4 ms 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 (CS\textsubscript{START}) is not consistent with offset facilitation, while the lack of substantial change in the falling phase of the delay function (CS\textsubscript{END}) is not consistent with sustained facilitation (Fig. 2).

An increased rise time (Fig. 4A) clearly shifted the CS\textsubscript{START} to longer delays, similar to the neuron in Figure 3.

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 Figures 5A and 5B, we plotted the timing of the leading edge of the delay function (CS\textsubscript{START}) for 4 ms vs 13 ms durations (Fig. 5A) and for 4 ms vs 31 ms durations (Fig. 5B). As described in Figure 2A, onset- and sustained-type facilitatory interactions show no change in CS\textsubscript{START} at different low frequency durations, corresponding to data scatter along the \textit{solid} diagonal line ($y = x$) in Figures 5A and 5B. Offset-type facilitatory interactions would show a shift in CS\textsubscript{START}, 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 \textit{dashed} diagonal lines in Figures 5A and 5B ($y=x+9$ 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 CS\textsubscript{START} measure that is consistent with offset-type facilitation.

In Figures 5C and 5D, we compared the timing of the trailing edge of the delay function (CS\textsubscript{END}). As described in Figure 2A, onset-type facilitation would result in no change in CS\textsubscript{END},
corresponding to data scatter along the *solid* diagonal lines in Figures 5C and 5D. In contrast, both sustained- and offset-type facilitation would result in an increase in CS\textsubscript{END}, corresponding to data scatter along the *dashed* diagonal lines in Figures 5C and 5D. Again, there is variability in this measure across duration, but no consistent shift in the CS\textsubscript{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 $5.3 \pm 3.7$ 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. 6A, 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\textsubscript{START} (mean change: $2.7 \pm 4.3$ ms, p < 0.001, paired t-test) and CS\textsubscript{END} (mean change: $5.7 \pm 5.4$ ms, p < 0.001, paired t-test). These results are consistent with the prediction in Figure 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 ms 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 ofSuppressive 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 Figure 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 $C_{\text{START}}$) and not significantly increased at longer durations (little or no change in $C_{\text{END}}$). 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 $C_{\text{START}}$) as the low frequency duration increased, but displayed a significant increase in $C_{\text{END}}$. 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 (Figs. 8A and 8B, 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 to suppression tuned below 23 kHz. Thus, 23-30 kHz suppression had lower thresholds and had different temporal properties when associated with excitation (Nataraj and Wenstrup, 2006). The present results reveal 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 23-30 kHz band showed less sensitivity to changes in duration (e.g., Figs. 7A, B) than did interactions tuned in the lower frequency band (e.g., Figs. 8A, B). This is illustrated for the sample of neurons with low frequency suppression in Figure 9, in which we plot the change in CSEND for 4 ms and 31 ms signals as a function of the tuning of the low frequency interaction. The large values for suppressive responses tuned in the 10-21 kHz range indicate that the suppression lengthens with signal duration. The small values of CSEND for suppressive responses tuned in the 22-31 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 10-22 kHz and 23-30 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 (CSSTART) and offset (CSEND) of suppression for different durations of low frequency sounds. This population analysis supports conclusions that low frequency suppression tuned below 23 kHz is different from low frequency suppression tuned in the range 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 CSSTART) that relates to frequency tuning of suppression or to the duration of the low frequency signal (Fig. 10A,B). The principal difference, instead, relates to the timing of the offset of suppression (CSEND). For neurons with suppression tuned < 23 kHz, the suppression significantly increased with longer duration stimuli (Fig. 10C,D, filled circles scattered along dashed diagonal line). In contrast, suppression tuned in the range 23-30 kHz changed little even
for longer duration, 31 ms signals (Fig. 10C,D, open circles 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,B). For suppression tuned below 23 kHz, the strength increased significantly as signal duration was increased beyond 4 ms. This was not the case for suppression tuned in the 23-30 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 Figure 2B. Thus, slower rise times shifted features of suppressive delay functions (both CS\text{START} and CS\text{END}) to significantly longer delays ($p < 0.01$, multiple paired $t$-tests, 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 displayed more than one of the types of spectral interactions described above. When a subset of neurons ($n = 17$) were systematically investigated for multi-tuned effects, 41% ($n = 7$) were found to exhibit sustained suppression tuned to frequencies below 23 kHz and phasic suppression ($n = 5$) or facilitation ($n = 3$) at or above 23 kHz. The neuron in Figure 12 displayed all three forms of low frequency interaction described in this report: 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 a phasic discharge, while 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 durations of 4 and 31 ms, both in terms of the delay function and the temporal response pattern (Fig. 12A, PSTHs). There was strong suppression of the BF response by the 27 kHz signal over a range of delays from -10 ms (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 appears to be independent of signal duration, since the delay function and temporal response patterns are nearly identical under the 4 ms and 31 ms conditions.

After completing two-tone tests with BF and 27 kHz signals, we then examined effects of 20 kHz tones on BF (Fig. 12B). At this time, the response to the BF signal doubled compared to 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.

Since we only examined the co-occurrence of such responses later in this study, the exact
proportions of neurons with suppression below 23 kHz and either facilitation or suppression in
the 23-30 kHz range is unclear. It is our sense, however, that suppressive effects of signals below
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 inferior colliculus (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 brainstem inputs that display 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 employed in this study.

Temporal Properties of Facilitation in Combination-Sensitive Neurons
Facilitation, referring to a non-linear 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 sub-millisecond range, and this selectivity appears 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 post-inhibitory rebound (Nataraj and Wenstrup, 2005; Sanchez et al., 2008; Wenstrup and Leroy, 2001), appear to interact independent of the glutamatergic inputs that establish the neuron’s response to sounds near its best frequency (Peterson et al., 2008; Sanchez et al., 2008). The present study shows 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, not by stimulus duration. This is followed by a rebound depolarization with timing also dependent on passive and active post-synaptic 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 brainstem 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 appear 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 Radtke-Schuller, 1987; Portfors and Wenstrup, 2001). While 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 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 (O’Neill and Suga, 1982; Olsen and Suga, 1991; Portfors and Wenstrup, 1999). However, both the mechanisms and their uses in acoustic analyses are more general. For example, the spectrotemporal integrative properties described 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; Ohlemiller 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 display two forms of low frequency suppression of responses to best frequency tones, apart from lower sideband inhibition. One form is characterized by responsiveness to frequencies in the bat’s audible range below 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 below 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 brainstem, 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, the present 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 due to primary focus on combination-sensitive inhibition in the 23-30 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 BF.
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 mechanics 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 (Holstrom 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 appears to depend on neural inhibition. The suppression is tuned to frequencies in the range 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). The present study shows 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 appear 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 glycinergic inhibition generally fails to eliminate suppression tuned in the 23-30 kHz (Nataraj and Wenstrup, 2005, 2006). In intracellular recordings from IC neurons that show suppression by 23-30 kHz sounds, the majority (57%) does not display low frequency-evoked inhibitory postsynaptic potentials (IPSPs) (Peterson et al., 2008). These results support a conclusion that combination-sensitive inhibition originates in auditory brainstem 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 brainstem 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 23-30 kHz inhibition is enhanced in some IC neurons by direct low frequency inhibitory input. Combination-sensitive inhibition thus appears to be a complex response property that arises through multiple, spectral 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 23-30 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 responses 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).

While 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 (versus 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 and Schreiner, 1999). These interactions contribute to analyses of a broad range of complex sounds.
ACKNOWLEDGEMENTS

This work was supported by grants R01 DC00937 (J.J.W.), and F32 DC007786 (D.C.P.) from the National Institute on Deafness and Other Communication Disorders of the U.S. Public Health Service. We thank W.E. O’Neill for discussions that initiated this study, the Auditory Neuroscience Group at NEOUCOM 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. Current address of Kianoush Sheykholeslami, M.D., Ph.D.: Department of Otolaryngology-Head and Neck Surgery, University Hospitals of Cleveland, Case Medical Center, Case Western Reserve University, 11100 Euclid Avenue, Cleveland, Ohio 44106.
LITERATURE CITED


Portfors CV, Felix RA 2nd. Spectral integration in the inferior colliculus of the CBA/CaJ mouse. 


FIGURE LEGENDS

Figure 1. Measures of frequency response and timing of facilitatory and suppressive spectral interactions. A. Responses of two neurons to BF tones when a second tone is varied in frequency. For both neurons, BF and low frequency tones were presented simultaneously. Neuron 1 shows a purely suppressive effect of the second tone in the range 16-21 kHz. Neuron 2 show suppression of the BF response by sounds in the range 12-20 kHz and facilitation of the BF response by sounds in the range 24-28 kHz. Neuron 1 sound levels: BF, 58 dB SPL, low frequency tone averages 70 dB SPL. Neuron 2 sound levels: BF, 17 dB SPL, low frequency tone averages 60 dB SPL. In B and C, “Delay curves” plot sensitivity of neurons to the relative timing of low frequency and best frequency (BF) signals. B. Facilitation is revealed by a peak in the delay function. C. Suppression is indicated by a trough in the delay function. “CSSTART” provides an estimate of the onset of the interaction, while “CSEND” provides an estimate of the offset of the facilitatory or suppressive interaction. See Materials and Methods for definitions. Error bars in B and C display the 95% confidence intervals of mean spike counts. These confidence intervals show that the response values in the peaks or troughs are significantly different from responses to either low or high frequency sounds presented alone.

Figure 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 grayered, 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. See Results for further description.
Figure 3. Temporal features of facilitation are unaffected by changes in duration of low frequency signals. High frequency signal at neuron’s BF: 71 kHz, 47 dB SPL, 4 ms duration. Low frequency facilitating signal set at 27 kHz, 45 dB SPL. Changes in low frequency duration had very little effect on delay functions; a change in low frequency rise time shifted the delay function to the right.

Figure 4. Facilitation delay functions of varying width are minimally affected by changes in low frequency temporal features. A. This neuron showed a very broad delay function that increased slightly between 4 ms and longer durations. High frequency signal at neuron’s BF: 58 kHz, 48 dB SPL, 4 ms duration. Low frequency facilitating signal set at 26 kHz, 73 dB SPL. B. A. The narrow delay function of this neuron increased slightly between 4 ms and longer durations. High frequency signal was at neuron’s BF: 58 kHz, 21 dB SPL, 4 ms duration. Low frequency facilitating signal set at 29 kHz, 62 dB SPL.

Figure 5. Measures of the timing of facilitation at different low frequency durations. Each data point (filled circle) compares values for one neuron: $\text{CS}_{\text{START}}$ for 4 ms vs 13 ms (A) or 4 ms vs 31 ms (B) low frequency durations and $\text{CS}_{\text{END}}$ for 4 ms vs 13 ms (C) or 4 ms vs 31 ms (D) low frequency durations. Symbols on solid line indicate that values were identical for the different duration stimuli. Symbols on the dashed line indicate that values for the longer sound were increased by the difference between the shorter and longer sound. Symbols adjacent to the horizontal and vertical axes indicate the mean and standard deviation for the corresponding measure. In all four plots, there was no significance difference in the measures at different durations (multiple paired $t$-tests).

Figure 6. Measures of the strength of facilitation at different low frequency durations and rise-fall times among neurons in the recorded sample. Each data point (filled or unfilled circle)
compares the interaction index (see Methods) for one neuron in two duration conditions: 4 ms vs 13 ms (A) or 4 ms vs 31 ms (B) low frequency durations, and for 4 ms (0.5 rise fall time) vs 13 ms (5 ms rise-fall time) (C). Filled symbols indicate neurons showing facilitation under both conditions. Unfilled symbols indicate neurons showing facilitation under one of the two conditions. Symbols adjacent to the horizontal and vertical axes indicate the mean and standard deviation for the corresponding measure. For each plot, there was no significance difference in the measures of facilitation strength at different durations or rise-time (multiple paired t-tests).

Figure 7. Suppression delay functions in two neurons showed little or no effect of changes in low frequency duration. A. Duration-insensitive suppression in neuron with no low frequency excitation. Brief suppression at delay = 0 ms was unaffected by low frequency duration. High frequency signal at neuron’s BF: 96 kHz, 55 dB SPL, 4 ms duration. Low frequency suppressing signal at 25 kHz, 51 dB SPL. B. In this neuron, displaying a suppression/excitation pattern in response to the low frequency signal, suppression delay functions showed little change with different low frequency durations, even though the magnitude of response to the low frequency signal changed significantly with duration. High frequency signal was at neuron’s BF: 76 kHz, 54 dB SPL, 4 ms duration. Low frequency facilitating signal set at 26 kHz, 56 dB SPL. Peristimulus time histograms show temporal pattern of spike discharge for the neuron in part B. Small rectangles above histograms show timing of low frequency (unfilled rectangles) and high frequency (filled rectangles) sounds. Numbers at lower right of each histogram shows the spike count occurring within a specified time window (grey rectangle).

Figure 8. Suppression delay functions in two neurons were significantly altered by changes in low frequency duration. A. In this neuron, low frequency signals evoked only
suppression. High frequency signal at neuron’s BF: 59 kHz, 55 dB SPL, 4 ms duration. Low
frequency suppressing signal at 18 kHz, 77 dB SPL. B. In this neuron, an excitatory response to
low frequency signals preceded the suppression. High frequency signal at neuron’s BF: 57 kHz,
32 dB SPL, 4 ms duration. Low frequency excitatory/suppressive signal at 17 kHz, 79 dB SPL.
Peristimulus time histograms show temporal pattern of spike discharge for the neuron in part B.
For protocol, see Figure 7 legend.

Figure 9. Changes in low frequency duration have different effects on features of
suppressive delay functions, depending the frequency of suppression. For suppressive
frequencies below 22-23 kHz, an increase in sound duration from 4 ms to 31 ms results in a large
change in CS\textsubscript{END}, indicating that suppression generally lasts for the duration of the low frequency
signal. For frequencies above 22-23 kHz, there is much less change in CS\textsubscript{END} as low frequency
duration increases from 4 ms to 31 ms.

Figure 10. Measures of the timing of low frequency suppression. Here we compare
timing for suppression tuned < 23 kHz (filled circles) and suppression tuned in the 23-30 kHz
range (open circles). A,B. Comparisons of CS\textsubscript{START} for 4 ms vs 13 ms (A) or 4 ms vs 31 ms (B)
low frequency durations. There were no significant differences in the onset of suppression
(CS\textsubscript{START}) for different duration stimuli (paired \textit{t}-tests) or for the different bands of low
frequency suppressive sounds (unpaired \textit{t}-tests). C,D. Comparisons of CS\textsubscript{END} for 4 ms vs 13 ms
(C) or 4 ms vs 31 ms (D) low frequency durations. For suppression evoked by sounds <23 kHz,
longer duration signals evoked significantly longer suppression, as indicated by the increasing
offset of suppression (CS\textsubscript{END}) for longer duration stimuli. For suppression evoked by 23-30 kHz
sounds, longer duration sounds slightly increased (C) or did not increase (D) the offset of
inhibition. At longer durations, the offset of suppression was significantly greater in the
population of neurons tuned to sounds $< 23$ kHz (C, D). As in Figure 5, symbols on solid line indicate that values were identical for the different duration stimuli. Symbols on the dashed line indicate that values for the longer sound were increased by the difference between the shorter and longer sound. Symbols adjacent to the horizontal and vertical axes indicate the mean and standard deviation for the corresponding measure. Thick curved lines/arrows in C and D show statistical comparisons using paired $t$-tests, while thick angled lines/arrows indicate results of unpaired $t$-tests (**, $p < 0.001$).

Figure 11. Measures of the strength of low frequency suppression. Each data point (filled or unfilled circle) compares the interaction index (see Methods) for one neuron under two duration conditions: 4 ms vs 13 ms (A) or 4 ms vs 31 ms (B) low frequency durations, and for 4 ms (0.5 rise fall time) vs 13 ms (5 ms rise-fall time) (C). Filled symbols indicate neurons with suppression tuned $< 23$ kHz. Unfilled symbols indicate neurons with suppression tuned in the range 23-30 kHz. Symbols adjacent to the horizontal and vertical axes indicate the mean and standard deviation. For suppression tuned $< 23$ kHz, increasing low frequency duration increased the strength of suppression (paired $t$-tests indicated by thick curved lines, **, $p < 0.001$). No similar change was observed for suppression tuned to 23-30 kHz. Strength of suppression for long duration low-frequency stimuli was greater for $< 23$ kHz signals than for 23-30 kHz signals (unpaired t-tests indicated by thick angled lines, *, $p < 0.01$). Rise-fall time had no effect on the strength of low frequency suppression.

Figure 12. A single IC neuron displays multiple spectral interactions with different temporal features. A. Facilitation and suppression tuned to 27 kHz were generally invariant with changes in duration of 27 kHz tone. Facilitation tuned to 27 kHz was maximum at a delay 18 ms. This neuron was noteworthy for the long latency of its response to BF and low frequency signals
(26 and 42 ms, respectively; see peristimulus time histograms). At delays of -10 ms to +4 ms, each signal suppresses the response to the other signal. At positive delays, there is a strong facilitatory interaction that is best at 16-20 ms. High frequency signal at neuron’s BF: 58 kHz, 37 dB SPL, 13 ms duration. Low frequency facilitating signal set at 42 dB SPL. B. For the same neuron, suppression tuned to 20 kHz lengthened as the duration of the 20 kHz signal increased. High frequency signal is the same as in part A; low frequency signal set at 63 dB SPL. In both A and B, spike counts were measured within a window centered on the response to the BF tone burst (grey rectangle in peristimulus time histograms), in order to evaluate low frequency facilitation or suppression of the BF response.
Figure 1

A

Individual Tones (kHz) vs. Delay of High Frequency Signal (ms)

Mean Spikes per Stimulus vs. Frequency of Second Tone (kHz)

Neuron 1 (BF=58 kHz)

Neuron 2 (BF=51 kHz)

B

Mean Spikes per Stimulus vs. Delay of High Frequency Signal (ms)

C

Mean Spikes per Stimulus vs. Delay of High Frequency Signal (ms)
Figure 2

A

Low Frequency Effect

Response to Combination

Delay Function

Onset Facilitation

Offset Facilitation

Sustained Facilitation

B

Low Frequency Effect

Response to Combination

Delay Function

Onset Facilitation

No Facilitation

CSSTART CS END

Delay of BF Signal (ms)

Delay Function

Delay of BF Signal (ms)

short r/f long r/f

long L short L
Figure 3

Low Frequency Duration
- 4 ms
- 13 ms
- 31 ms
- 13 ms / 5 ms rise

Spikes per 32 Stimuli

Delay of High Frequency Signal (ms)

Individual Tones (kHz)
Figure 7

A

Spike per 32 Stimuli

Delay of High Frequency Signal (ms)

Individual Tones (kHz)

B

Spike per 32 Stimuli

Delay of High Frequency Signal (ms)

Individual Tones (kHz)

76 kHz

26 kHz / 4 ms, Δt = -2 ms

Δt = 0 ms

Δt = 10 ms

Δt = 28 ms

26 kHz / 13 ms

35

35

35

26 kHz / 31 ms

10

32

14

33
Figure 8

**A**

Low Frequency Duration

- **4 ms**
- **13 ms**
- **31 ms**

Spikes per 32 Stimuli

Delay of High Frequency Signal (ms)

Individual Tones (kHz)

---

**B**

Spikes per 32 Stimuli

Delay of High Frequency Signal (ms)

Individual Tones (kHz)

---

**C**

Spikes

Time (ms)

---

**D**

Spikes

57 kHz

17 kHz / 4 ms

Δt = 0 ms

Δt = 6 ms

Δt = 18 ms

Δt = 38 ms

17 kHz / 13 ms

17 kHz / 31 ms

---

**E**

Spikes

32

16

0

0 75 0 75 0 75 0 75 0 75 0 75 0 75
Figure 9

The graph shows the relationship between the frequency of suppression (kHz) and the change in CSEND (ms). The data points represent individual neurons, while the line indicates the average value (2 kHz wide). The x-axis represents the frequency of suppression, and the y-axis represents the change in CSEND.
Figure 10

**Figure 10**

(A) Scatter plot showing CS\(_{START}\) for 13 ms signal (ms) against CS\(_{START}\) for 4 ms signal (ms) for LF < 23 kHz (filled circle) and LF ≥ 23 kHz (open circle).

(B) Similar plot for CS\(_{START}\) for 31 ms signal (ms) against CS\(_{START}\) for 4 ms signal (ms).

(C) Scatter plot showing CS\(_{END}\) for 13 ms signal (ms) against CS\(_{END}\) for 4 ms signal (ms) with significant differences indicated by "**".

(D) Similar plot for CS\(_{END}\) for 31 ms signal (ms) against CS\(_{END}\) for 4 ms signal (ms) with significant differences indicated by "**".
Figure 11

A

Index for 13 ms duration

Index for 13 ms (5 ms RF) duration

B

C

Index for 31 ms duration

Index for 4 ms duration