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ROLES OF INHIBITION IN CREATING COMPLEX AUDITORY RESPONSES IN THE INFERIOR COLLICULUS: FACILITATED COMBINATION-SENSITIVE NEURONS

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ABSTRACT

We studied roles of inhibition on temporally sensitive facilitation in combinationsensitive neurons from the mustached bat's inferior colliculus (IC). In these integrative neurons, excitatory responses to best frequency (BF) tones are enhanced by much lower frequency signals presented in a specific temporal relationship. Most facilitated neurons (76%) showed inhibition at delays earlier than or later than the delays causing facilitation. The timing of inhibition at earlier delays was closely related to the best delay of facilitation, but the inhibition had little influence on the duration or strength of the facilitatory interaction. Local iontophoretic application of antagonists to receptors for glycine (strychnine, STRY) and γ -aminobutyric acid (GABA) (bicuculline, BIC) showed that STRY abolished facilitation in 96% of tested units, but BIC eliminated facilitation in only 28%. This suggests that facilitatory interactions are created in IC, and reveals a differential role for these neurotransmitters. The facilitation may be created by coincidence of a postinhibitory rebound excitation activated by the low frequency signal with the BF-evoked excitation. Unlike facilitation, inhibition at earlier delays was not eliminated by application of antagonists, suggesting an origin in lower brainstem nuclei. However, inhibition at delays later than facilitation, like facilitation itself, appears to originate within IC and to be more dependent on glycinergic than GABAergic mechanisms. Facilitatory and inhibitory interactions displayed by these combination-sensitive neurons encode information within sonar echoes and social vocalizations. The results indicate that these complex response properties arise through a series of neural interactions in the auditory brainstem and midbrain.

Key Words: combination-sensitive; delay-tuned, inferior colliculus, mustached bat, spectral integration

INTRODUCTION

Inhibition plays multiple roles in determining the output of sensory neurons. These roles may be particularly varied in the auditory system, because, in addition to intrinsic interactions within nuclei, the ascending pathway features inhibitory projection neurons targeting auditory brainstem, midbrain, and thalamic nuclei (Adams and Mugnaini 1984, 1990; Saint Marie et al. 1989; Winer et al. 1995, 1996). Inhibition determines basic auditory response properties such as evoked discharge rates (Backoff et al. 1999; Foote et al. 1975; Wang et al. 2000), temporal response patterns (Caspary et al. 1979; Chen and Jen 2000; LeBeau et al. 1996; Pollak and Park 1993), responsiveness as a function of sound level (Chen and Jen 2000; Yang et al. 1992; Vater et al. 1992), and frequency response areas (Fuzesserv and Hall 1996; LeBeau et al. 2001; Yang et al. 1992). Inhibition also plays major roles in the creation of more complex responses. In the inferior colliculus (IC), for example, many complex response properties arise *de novo* through specific inhibitory input that creates or enhances selectivity for certain stimulus features. GABAergic inhibition contributes to directional selectivity to frequency modulated sweeps (Fuzessery and Hall 1996) and to selectivity for binaural cues (Faingold et al. 1991; Fujita and Konishi 1991; Park and Pollak 1993). Both GABAergic and glycinergic mechanisms mediate duration tuning (Casseday et al. 1994). In each of these examples, inhibition appears to have frequency tuning, aurality, or timing that is different from the dominant excitatory input. Understanding the inhibition is essential for understanding the mechanisms that create the response selectivity.

This study analyzes the role of inhibition among neurons of the IC that show temporally sensitive facilitation to the combination of acoustic elements in vocalizations. In the mustached bat, these combination-sensitive neurons are abundant in the auditory forebrain (Fitzpatrick et al.

1998; Olhemiller et al. 1996; Suga et al. 1978, 1983), but also in the IC where they appear to originate (Mittmann and Wenstrup 1995; Portfors and Wenstrup 1999; Wenstrup and Leroy 2001). This integration of distinct spectral sensitivities appears to depend on specific low frequency-tuned inputs to high frequency regions of the IC (Wenstrup et al., 1999). Inhibition is associated with these facilitated responses, both in terms of the response to the relative timing of different signal elements (Edamatsu and Suga 1993; Olsen and Suga 1991b; Portfors and Wenstrup 1999), and in terms of the underlying mechanisms (Wenstrup and Leroy 2001). While it is clear that inhibitory interactions accompany facilitation in the IC, medial geniculate body, and auditory cortex, it is not clear how the inhibition and facilitation are related in IC neurons, nor is it clear where the inhibitory interactions originate. In the present study, we related inhibitory and facilitatory interactions across a sample of facilitated combination-sensitive IC neurons, and we blocked receptors to the inhibitory neurotransmitters glycine and γ aminobutyric acid (GABA). The results show that glycinergic input to the IC is essential for the facilitatory interactions, but GABAergic input is not required. Moreover, while inhibitory interactions appear to affect the timing of the facilitatory interaction, blockade of the receptors indicates that some of the inhibitory combination-sensitive interactions originate at levels below the inferior colliculus.

MATERIALS AND METHODS

We examined auditory responses of single units from the inferior colliculus (IC) of awake mustached bats (*Pteronotus parnellii*) captured in Trinidad and Tobago. Procedures used in this study were approved by the Institutional Animal Care and Use Committees at the Northeastern Ohio Universities College of Medicine and The University of Akron.

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Surgical procedures

Twelve bats provided data used in this study. Some bats were anesthetized with methoxyflurane as described previously (Portfors and Wenstrup 1999), but most were anesthetized with isoflurane (1.5–2.0%; Abbott Laboratories, North Chicago, IL). Ten minutes prior to isoflurane application, the animal received intraperitoneal injections of butorphanol (5 mg/kg, Fort Dodge Animal Health, Fort Dodge, IA) and atropine (0.06 mg/kg, Phoenix Scientific Inc, St Joseph, MO). After the anesthetic abolished nociceptive reflexes, the dorsal surface of the head was shaved and cleaned. A midline incision was made in the skin and the underlying muscles were reflected laterally to expose the skull. The skull surface was cleaned and a tungsten ground electrode was cemented into the lateral surface of the skull overlying the cerebral cortex. A metal pin was cemented to the skull to secure the head in the stereotaxic apparatus during physiological experiments. A hole (usually < 0.5 mm in diameter) was placed in the skull above the IC. After these procedures were completed, a local anesthetic (Lidocaine) was applied to the surgical areas and the animal was returned to the holding cage. The bat recovered for 2-3 days before starting physiological experiments.

Acoustic stimulation and data acquisition

Acoustic stimulation was computer-controlled. Single and multiple tone bursts (4 to 31ms duration, 0.5 ms rise/fall time, 4 presentations per second) were computer synthesized, downloaded to a digital signal processor (Tucker-Davis Technologies, model AP2), then converted to analog signals at a sampling rate of 500 kHz (Tucker-Davis Technologies, model DA3-2). The analog signal was filtered (Tucker-Davis Technologies, model FT6-2), attenuated (Tucker-Davis Technologies, model PA4), and then sent to a power amplifier (Parasound model

HCA-800II) and the loudspeaker (Technics Leaf Tweeter, model EAS 400B). The speaker was placed 10 cm in front of the bat and 25° into the sound field contralateral to the IC under study.

Extracellular action potentials were amplified, filtered (bandpass, 500-6000 Hz), and sent through a spike signal enhancer (Fredrick Haer and Co., model 40-46-1) before being digitized at a sampling rate of 40 kHz (Tucker-Davis Technologies, model AD2). The digitized signal was uploaded to the computer via a second AP2 digital signal processing card. Custom software calculated the time of occurrence of the spikes and displayed peristimulus time histograms (PSTHs), raster plots, and statistics of the neural responses in real time.

Recording procedures

Physiological experiments were conducted inside a single-walled acoustic chamber lined with polyurethane foam to reduce echoes. On experimental days, the bat was placed in a stereotaxic apparatus inside the heated and humidified acoustic chamber. If the bat showed signs of discomfort or distress, it was lightly sedated with acepromazine (1 mg/kg, subcutaneous) or butorphanol (0.05 mg/kg, subcutaneous), or removed from the stereotaxic apparatus for the day. The bat was offered water from a medicine dropper between electrode penetrations. Recording sessions did not exceed 4-6 hours and were limited to one per day.

Single-unit recordings were obtained using a glass micropipette electrode mounted on a 5-barreled pipette (Havey and Caspary 1980). The multibarreled pipette (A-M Systems Inc.) was pulled to a tip and broken to a total tip diameter of 15-30 μ m. The single barrel recording electrode (A-M Systems Inc.) was glued onto the tip of the multibarrel pipette at a 20° angle and protruded 10-25 μ m beyond it. The recording electrode was filled with 1 M NaCl and had resistances in the 10–30 M Ω range. One barrel of the pipette, filled with 1M NaCl, balanced currents of other channels that delivered or retained drugs. The remaining barrels were filled

with strychnine-HCl (STRY) (10 mM in distilled water, 3.0 pH, Fluka, Milwaukee, WI), and bicuculline-methiodide (BIC) (10 mM in 0.9% NaCl, 3.0 pH, Sigma, St. Louis, MO). All drugs and recording solutions were prepared on the day of the experiment. Both strychnine and bicuculline-methiodide were retained with negative current (-15 nA each) and ejected using positive currents (15-40 nA for strychnine and 10-50 nA for bicuculline). Each barrel of the multibarreled pipette was connected via a silver wire to a channel of a microiontophoresis current generator (Dagan Programmable current generator, model 6400). The current generator controlled retention and ejection currents for each barrel separately. Drug ejection times and ejection currents varied depending on the drug and the effect of the drug, as evaluated audiovisually and by quantitative tests.

Electrodes were angled 10–20° (dorsolateral to ventromedial) to record from tonotopic regions of the central nucleus of the inferior colliculus (ICC) representing higher frequencies (56–120 kHz), and advanced by a hydraulic micropositioner (David Kopf Instruments, model 650).

Data were obtained only from well isolated single units. When a single unit was isolated, the best frequency (BF) (defined as that frequency requiring the lowest sound level to elicit consistent, stimulus-locked action potentials) and minimum threshold (MT) at best frequency (defined as the lowest sound level at the best frequency to elicit consistent, stimulus-locked action potentials) were obtained with single tone burst stimuli. This use of the term "best frequency", common in the neuroethological literature, is equivalent to "characteristic frequency". Since many IC neurons respond best to the combination of distinct spectral elements in sounds (combination sensitivity), we used a two-tone stimulus paradigm to evaluate the underlying neural interactions. One of the tones was at the unit's BF (high frequency signal), 10

dB above threshold. The second signal (low frequency signal), tuned to lower frequencies within (23-30 kHz) or adjacent to the first harmonic of the biosonar call, was varied over a range of frequencies, sound intensities, and timing (delays) relative to the BF signal. When a combination-sensitive response was suspected, quantitative measures of delay-sensitive facilitation or inhibition were obtained and compared to single-tone responses. Audiovisual examination established the range of delays over which quantitative data were obtained. Delay sensitivity was evaluated in 2 ms steps. Typically, very short signals (4 ms) were used in order to reveal maximum temporal sensitivity; longer duration stimuli were only used if units were unresponsive to 4 ms signals. Responses to 32 presentations of each stimulus were collected.

Units were considered to show facilitatory combination-sensitive interactions if the response to the combined low and high frequency signals, separated by the appropriate delay, was at least 20% higher than the sum of the responses to the two signals presented separately (20% facilitation). Similarly, units were considered to show inhibitory combination-sensitive interactions if the response to the combined low and high frequency signals, separated by the appropriate delay, was at least 20% lower than the sum of the responses to the two signals presented separately (20% inhibition). The strength of combination-sensitive interactions was quantified by an interaction index, where *index* = (Rc-Rl-Rh)/(Rc+Rl+Rh). Rc is the unit's response to the combination of low and high frequency signals, Rl is the unit's response to the low frequency signal alone, and Rh is the unit's response to the high frequency signal alone. Interaction index values of 1.09 and -0.11 correspond to thresholds for facilitation and inhibition, respectively. In some units, high background discharge affected

calculation of the interaction index. This effect was minimized by analyzing responses within a 50-60 ms window.

Delay sensitivity tests and rate-level functions were obtained prior, during and in some cases, after application of drugs. During drug application, rate-level or delay sensitivity tests were continuously obtained. Changes in response magnitude or the shape of response functions served to indicate the presence of a drug near the recording site. Low ejection currents, e.g. 10 nA, were used initially. If no effect was observed, the ejection current was gradually increased. For every current setting, delay curves were obtained until no further changes in response magnitudes or response functions were observed. With currents used in this study, effects of BIC and STRY could be observed as early as 2 and 3 minutes, respectively, while steady state effects of BIC and STRY could be observed after 4 and 8 minutes, respectively. If there was any indication that drugs could not be ejected, the pipette/electrode assembly was retracted and discarded. New piggy-backed electrodes were used for every penetration.

Identical tests were performed before applying a drug (Control), during drug application (Drug) and after drug application stopped (Recovery). A unit was considered to show complete recovery when response functions and response magnitude returned to control values. Recovery could be observed as early as 15 minutes or as long as 45 minutes after drug application was stopped, and was dependent on the particular drug, ejection currents, and the duration of drug application. To save time, data were collected in the following sequence: Control, Drug-1, Drug-1+Drug-2, Recovery, Drug-2, and Recovery. The two drugs were STRY and BIC and were, interchangeably, applied as Drug-1 or Drug-2.

Data analysis

Combination-sensitive interactions, facilitatory or inhibitory, were characterized by three features: 1) the delay at which the interaction was at its maximum (best delay), 2) the range of delays over which the interaction occurred (delay width), and 3) the maximum strength of the interaction (interaction index). Figure 1 illustrates these features for a unit that was both facilitated and inhibited.

Statistical analyses were performed with an error (α) level of 0.05. Analyses of variance tested for differences in various features of combination-sensitive interactions (best delay, delay width, interaction index) across the groups of facilitatory units. Within each group, paired t-tests were used to find significant differences among the features of the combination-sensitive interactions and regression analyses were performed to find significant correlations between these features. Mean values are reported with the corresponding standard error (mean \pm standard error of the mean).

RESULTS

This study is based on recordings of 346 single units that were characterized by the effects of two-tone acoustic stimulation. Of these units, 80 (23%) displayed combinationsensitive facilitation. That is, the response to BF tones was enhanced by a second, lower frequency tone presented in a specific temporal relationship. BFs of the units were tuned between 58.2 and 109.4 kHz, the upper half of the animal's audible range. The best lower facilitating frequency was tuned between 20.3 and 38.8 kHz. The relative timing of the two signals that evoked the strongest facilitation (the best facilitatory delay) ranged from -3 to 30 ms, with positive values indicating that the higher frequency sound followed the lower frequency sound. For nearly all units (92%, n = 74), the frequency combinations and temporal relationships

that evoked facilitation occur in pulse-echo combinations of the biosonar signal. Some of these combinations also occur in social vocalizations. In six units the frequency combinations do not occur in sonar pulse-echo combinations.

Most of these facilitated units (76%, n = 58) also showed combination-sensitive inhibition at delays earlier and/or later than the delays causing facilitation. The Results describe the contributions of inhibition to the facilitatory interaction in two ways. Across the population of single units, we studied the relationships between features of inhibition and features of facilitation. In a subset of the units (n = 47), we further examined the effect of blocking glycinergic and/or GABAergic inhibition by local iontophoretic application of strychnine (STRY) or bicuculline (BIC), respectively.

Features of facilitatory and inhibitory interactions

Two-tone delay curves revealed the time-dependent facilitatory and inhibitory influences of low frequency signals on the responses to the higher, BF signals (Fig. 2). Figure 2A shows the response of a facilitated unit with no apparent inhibition at earlier or later delays. We observed a strong response to the BF tone (86.3 kHz), but a weak response to a low frequency tone (29.2 kHz). The strongest facilitation occurred when the two tones were presented simultaneously, at 0 ms delay. The absence of inhibition in the delay function suggests that the low frequency signal evoked only excitation. Figure 2B shows the response of a facilitated combination-sensitive unit with inhibition only at early delays. There was a moderate response to the BF tone (72.6 kHz) but a weak response to a low frequency tone (24.3 kHz). The strongest facilitation occurred when the BF tone was presented 12 ms after the low frequency tone. Strong inhibition occurred when the BF tone was presented 0–4 ms after the low frequency tone, with complete elimination of the response at 0 and 4 ms delay. This pattern suggests that the low frequency signal activated

inhibition followed by excitation. Figure 2C shows the response of a facilitated combinationsensitive unit with inhibition only at later delays. We observed a moderate response to the BF tone (72.1 kHz) but no response to the low frequency tone (26.8 kHz). The strongest facilitation occurred when the BF tone was presented 2 ms after the low frequency tone. Inhibition was evident when the BF tone was presented 8–10 ms after the low frequency tone (Fig. 2C). The pattern displayed by this unit suggests that the low frequency signal activated excitation followed by inhibition. Figure 2D shows the response of a facilitated combination-sensitive unit with both inhibition at early delays and inhibition at later delays. There was a moderate response to the BF tone (81.6 kHz) but no response to the low frequency tone (23.6 kHz). The strongest facilitation occurred when the BF tone was presented 4 ms after the low frequency tone. Suppression at early delays occurred when the BF tone was presented 0–2 ms before the low frequency tone. Suppression at later delays occurred when the BF tone was presented 12–16 ms after the low frequency tone. For these units, the delay curve suggests that the low frequency signal activated, in turn, inhibition, excitation, and inhibition.

The occurrence of these types of facilitated delay curves within the population is illustrated in Figure 3. Of 76 units for which a determination could be made, 24% showed no evidence of inhibition. Thirty units displayed only early inhibition, thirteen units showed only later inhibition, while 20% of the units showed both early and later inhibition. Overall, more units showed inhibition at early delays (59%) than at later delays (37%).

Among the 80 units analyzed, facilitatory interactions were limited within well-defined ranges. Thus, 83% were tuned to best delays between 0 and 10 ms (Fig. 4A) and 87% had delay widths in the 0–10 ms range (Fig. 4A). Most of the units (86%) had facilitation index values between 0.11 and 0.49 (Fig. 4A), corresponding to facilitatory increases in response magnitude

of 25–192%. We found significant correlations between the best delay and delay width (r = 0.25, p < 0.05), the best delay and strength of facilitation (r = 0.25, p < 0.05), and the delay width and strength of facilitation (r = 0.32, p < 0.01). However, these correlations do not indicate strong coupling between these features of facilitation.

Among the 45 facilitated units showing inhibition at early delays (Fig. 4B), most (66%) had a best inhibitory delay at 0 ms (Fig. 4B). The width of inhibition at early delays ranged between 1 ms and 13ms for nearly all neurons (91%, Fig. 4B). Across this population, the strength of inhibition at early delays was evenly distributed from -0.15 to -1.00 (Fig. 4B). The only significant correlation among the features of inhibition at early delays was between the best inhibitory delay and the width of the inhibition (r = 0.49, p < 0.01).

Twenty-three units showed inhibition at later delays. The best inhibitory delay ranged from 6 ms to 28 ms (Fig. 4C). The width of inhibition at later delays, measured in 19 units, ranged from 2-15 ms (Fig. 4C). The strength of inhibition at later delays ranged from -0.17 to -0.75. There were no significant correlations among the features of inhibition at later delays.

As expected, the best later inhibitory delays were longer than the best early inhibitory delays (f [2, 144] = 53.2, p < 0.001). Inhibition at early delays was stronger than at later delays (f [2, 143] = 323.1, p < 0.001), but delay width, a measure of the duration of the inhibition, was the same (f [2, 127] = 5.9, p > 0.1). The width of inhibition at early or later delays was greater than the width of facilitation (f [2, 127] = 5.9, p < 0.01).

Relationship between inhibition and facilitation

How do inhibitory interactions influence the features of combination-sensitive facilitation? In this section, we examine whether features of facilitation differ among combination-sensitive units based on the presence and timing of inhibition, and whether

facilitatory features are correlated with the timing, duration, or strength of inhibitory interactions among units showing these interactions.

There was a clear relationship between early inhibition and the best facilitatory delay. Most units without early inhibition (83%, n = 26) had best facilitatory delays of 4 ms or less (range: -3 to 10 ms; average: 2.4 ± 0.5 ms). In contrast, nearly all units (98%, n = 44) with early inhibition had best facilitatory delays of 4 ms or more (range: 0 to 30 ms; average: 9.1 ± 0.9 ms). This difference is highly significant (*f* [1.74] = 31.4, *p* < 0.001), suggesting that inhibition at early delays alters the best facilitatory delay of units. Moreover, among the 44 units showing inhibition at early delays, there was a positive correlation between the best early inhibitory delay and the best facilitatory delay (*r* = 0.63, *p* < 0.01, Fig. 5A). An even stronger correlation existed between the width of inhibition at early delays and the best facilitatory delay (*r* = 0.87, *p* < 0.01, Fig. 5B). The strength of inhibition at early delays was not significantly correlated with any measure of delay-tuned facilitation. This suggests that timing of inhibition at early delays, but not its strength, can affect the best delay of facilitation.

In contrast, the presence of inhibition at later delays had little influence on the best facilitatory delays of units. Specifically, the best facilitatory delays for units with inhibition at later delays were not significantly different from units that had no inhibition at later delays (f [1,74] = 0.52, p > 0.1). However, it is noteworthy that all units with best facilitatory delays greater than 15 ms had no inhibition at later delays.

Surprisingly, there was little evidence that the width of the facilitated delay curve was influenced by early or later inhibition. Thus, there was no significant difference in facilitatory delay width among units without inhibition, units with inhibition at early delays, and units with inhibition at later delays (f [2, 86] = 0.6, p > 0.5). No difference existed even between units

lacking inhibition and units having both early and later inhibition. Further, there was no significant correlation between any measure of early inhibition and the delay width of facilitation. These analyses suggest that the duration of delay-tuned facilitation, expressed as the delay width of facilitation, is not limited by early or later inhibition. However, it is noteworthy that the range of facilitatory delay widths for units without inhibition at later delays was broader (0.4 - 19.7 ms) than the range of facilitatory delay widths for units with inhibition at later delays (0.6 to 11.8 ms). This suggests that inhibition occurring at delays greater than the best facilitatory delay could potentially limit the width of long-duration facilitatory interactions, but did not have this effect on most units.

There were no significant differences in the strength of facilitatory interactions among units without inhibition, units with inhibition at early delays, and units with inhibition at later delays (f [2, 88] = 0.1, p > 0.5). Moreover, there was no significant correlation between the strength of facilitation and any feature of early inhibition. Thus, early inhibition had no measurable effect on the strength of the facilitatory interaction.

Since inhibition at later delays, by definition, occurs at delays later than both the best facilitatory delay and the best early inhibitory delay, we found significant correlations between: 1) the best facilitatory delay and best later inhibitory delay (r = 0.84, p < 0.01), 2) the best early inhibitory delay and best later inhibitory delay (r = 0.72, p < 0.05), and 3) the width of inhibition at early delays and best later inhibitory delay (r = 0.8, p < 0.05). The strength of inhibition at later delays was not significantly correlated with either the best facilitatory delay (r = 0.10, p > 0.1) or the strength of facilitation (r = 0.15, p > 0.1). However, the width of inhibition at later delays was significantly correlated with the delay width of facilitation (r = -0.53, p < 0.05, Fig.

5C) and strength of facilitation (r = -0.50, p < 0.05, Fig. 5D). This suggests that the inhibition at later delays depends to some extent on the strength and timing of the facilitatory interaction. *Effect of drugs on delay-tuned combination-sensitive responses*

We tested the role of glycine and GABA in facilitatory combination-sensitive units by examining their response properties before and during the application of strychnine (STRY: n = 23), bicuculline (BIC: n = 25), or both (BIC+STRY: n = 20).

Application of STRY eliminated some but not all features of the delay-sensitive response to combinations of tones. For the unit in Figure 6A, the facilitatory peak, occurring in control tests at delays centered at 10 ms, was completely eliminated. However, the inhibition at 0 ms delay was unaffected (control interaction index: –0.65, STRY: -0.63). Figure 6B shows the effect of STRY in a facilitated unit in which the lower frequency facilitating sound was tuned to frequencies outside the sonar range. The unit, recorded in the 80-90 kHz tonotopic representation of the IC, displayed two excitatory responses tuned to 20.3 kHz and 85.6 kHz. When the two signals were presented simultaneously (best delay = 0 ms), the response of the unit was facilitated. Application of STRY eliminated the facilitation (Fig 6B).

Application of BIC had less effect on delay-sensitive responses than did STRY. For the unit in Figure 7A, the response to the BF tone, at 81.6 kHz, was facilitated by a 23.6 kHz signal at a best delay of 6 ms. This unit also showed inhibition at both early and later delays. Application of BIC to the unit in Figure 7A did not eliminate either the delay-tuned facilitation or the inhibitory interactions at early delays. In fact, the strength of facilitation increased (facilitation index: Control: 0.2, BIC: 0.39). The failure to eliminate these interactions occurred even though BIC clearly affected the unit by increasing its response to the higher frequency sound. The addition of STRY to the BIC application eliminated the facilitatory peak evident in

the BIC curve. Inhibition at early delays was reduced further, but not eliminated. Figure 7B shows the effect of BIC on a facilitatory combination-sensitive unit, tuned to 37.8 kHz and 78.4 kHz. Application of BIC did not eliminate facilitation even though it significantly increased the response rate of the unit (Fig 7B).

Most units in our sample were tuned to frequency combinations characteristic of sonar signals (Fig 6A, 7A). In four units, however, the facilitatory interactions were tuned to frequency combinations that could occur in social vocalizations but not in sonar signals (Fig 6B, 7B). Among these units, the effects of STRY or BIC application were identical to those in the broader sample. In the unit where application of BIC did not eliminate facilitation, addition of STRY eliminated facilitation. Although the number of units is limited, the results suggest that non-sonar facilitatory interactions employ the same neural mechanisms used by neurons responding to frequency combinations in sonar signals.

Table 1 provides a general summary of the effects of STRY and BIC on facilitation and the accompanying inhibition at early and later delays. Facilitation was strongly dependent on glycinergic inhibition but much less dependent on GABAergic inhibition. STRY eliminated facilitation in 96% of the units when applied alone, and in 100% of the units when applied in combination with BIC. The loss of facilitatory interactions with application of STRY is consistent with earlier results (Wenstrup and Leroy, 2001). In contrast, BIC eliminated facilitation in 28% of the units. In most units, inhibition at early delays was not eliminated by STRY, BIC, or BIC+STRY. Inhibition at later delays was largely dependent on glycinergic inhibition. Thus, STRY eliminated later inhibition in 78% of tested units and BIC+STRY eliminated inhibition in 83% of tested units, while BIC eliminated later inhibition in only 20% of

the units. Below, we quantitatively assess the effects of the drugs on facilitation and the accompanying inhibition.

Quantitative effects of drugs on facilitation

Application of STRY eliminated facilitation in 22 of 23 units tested (Table 1), and severely reduced the strength of facilitation in the remaining unit (change in interaction index from 0.76 to 0.12, Fig 8A). Across the sample, the average strength of facilitation was significantly reduced (Control mean: 0.27, STRY mean: -0.03; p < 0.001, paired *t* test). In 5 units, the elimination of facilitation revealed an underlying inhibitory interaction at the best delay.

STRY also typically increased the response rate to the combination stimulus at best delay (Fig. 8D) and to the BF signal alone (Fig. 8G), by 27% and 137% respectively (p < 0.05, paired *t* test). These response increases might suggest that the loss of temporally sensitive facilitatory interactions resulted from saturation of the unit's response, such that the lower frequency facilitative signal could evoke no additional response above the BF response. If so, one might expect a significant correlation between the change in facilitation strength and the change in the magnitude of the combination or BF responses, due to application of STRY. However, there was no significant correlation in the change in the facilitation strength with changes in either the response to BF stimulus (r = 0.15; p > 0.05), or the response to the combination of sounds at best facilitatory delay (r = 0.03; p > 0.05). This analysis suggests that rate saturation is not the primary explanation for the loss of facilitation during application of STRY. Another way to evaluate saturation effects is to compare response rates at the best facilitatory delay with the rate-level function in response to the BF signal. If the maximum response rate in the rate-level functions exceeds the maximum response rate observed in the delay curve, then the unit could

potentially display a facilitatory response to the combination stimulus, despite the drug-induced increase in the overall response rate. In 7 of 7 units where rate-level functions could be obtained after STRY application, the loss of facilitation did not appear to result from rate saturation effects.

Of 25 units tested with BIC application, facilitation was eliminated in 7 units (Table 1). Across the sample, the strength of facilitation was significantly reduced (Control mean: 0.32, BIC mean: 0.16; p < 0.01, paired t test), but the mean for BIC application nonetheless corresponded to facilitation. For the 18 units in which BIC did not eliminate facilitation, the mean change in interaction index was only 0.06, corresponding to a change in facilitatory strength of about 13% (Control mean: 0.33, BIC mean: 0.27; p < 0.01, paired t test). Application of BIC significantly increased the response magnitudes of units, both to the combination stimulus at best delay (average increase, 82%) and to the BF signal alone (181%) (p < 0.05, paired t test, Fig 8E, 8H). For units tested with BIC, there was no significant correlation between the change in facilitation strength and changes in either the response to the BF stimulus (r = -0.38; p > 0.05) or the response to the combination stimulus at best facilitatory delay (r = -0.13; p > 0.05). Of the 7 units in which BIC eliminated facilitation, rate-level functions were obtained in 4 units with BIC. In 3 of the 4 units, a comparison of the rate-level and delay functions suggested that the loss of facilitation was not due to saturation effects. In the rest of the units, we could not determine whether the loss of delay-tuned facilitation is due to application of BIC or due to saturation of the unit's response. Despite the changes in response rates, other measures of combination-sensitive facilitation were not affected. For the 18 units in which BIC did not eliminate facilitation, BIC did not alter the best facilitatory delay (p > 0.1, paired t test, Fig. 9A) or the range of delays over which facilitation occurred (delay width: Control mean: 6.9 ms, BIC:

6.9 ms, p > 0.1, paired *t* test, Fig. 9B). These data suggest that the features of facilitatory combination-sensitive interactions are generally independent of GABAergic inhibition.

In all 20 units tested with combined application of BIC and STRY, facilitation was eliminated (Table 1) and the average strength of facilitation was significantly reduced (Control mean: 0.29, BIC+STRY mean: -0.04; p < 0.01, paired t test, Fig. 8C). For 6 units in which facilitation was eliminated by BIC+STRY, the loss of facilitation revealed an underlying inhibition at the best facilitatory delay. The average responses to the combination of sounds at best facilitatory delay and to the BF sound were significantly increased by 130% and 340%, respectively, due to application of BIC+STRY (p < 0.05, paired t test, Fig 8F, 8I). However, there was no significant correlation between the change in the facilitation strength and the change in the BF response (r = -0.13; p > 0.05), or with the change in response to the combination of sounds at best facilitatory delay (r = -0.24; p > 0.05). In all five units for which rate-level functions could be obtained, rate saturation did not appear to be a factor. In 9 facilitatory combination-sensitive units, STRY was applied first and then BIC was added. In all 9 units, STRY eliminated the facilitatory interaction and addition of BIC did not reverse the effect of STRY by itself. In another 9 facilitatory combination-sensitive units, BIC was applied first and then STRY was added. In 8 of these units (89%) BIC alone did not eliminate the facilitation, but addition of STRY eliminated the facilitation. The independent effect of STRY, in the presence or absence of BIC, supports a conclusion that facilitatory combination-sensitive interactions are more dependent on glycinergic inhibition than on GABAergic inhibition. Application of BIC+STRY allowed us to test the plausibility of the hypothesis that facilitation could result from disinhibition involving both GABA_A and glycine receptors. One model predicts that facilitation is retained when BIC and STRY are applied together. Application of

BIC+STRY eliminated facilitation in 100% of the units tested, suggesting that such a model of disinhibition does not underlie facilitatory combination-sensitive interactions (see Discussion). *Quantitative effects of drugs on inhibitory interactions*

Inhibition at early delays. The effect of drug application on the strength and the duration of early inhibition was tested in 30 units. In all 15 units tested with STRY, inhibition at early delays was not eliminated (Table 1). However, the average strength of inhibition, expressed as the interaction index, was significantly reduced from -0.62 to -0.41 (p < 0.01, paired t test, Fig. 10A). In somewhat over half of the units (60%, n = 9), the strength of inhibition decreased by >20%, but inhibitory strength was relatively unchanged in the remainder. In 10 of the units tested with STRY, the delay width of inhibition at early delays could be measured. There was no significant reduction in this inhibitory delay width due to application of STRY (Control mean: 10.9 ms, STRY mean: 7.7 ms, p > 0.05, paired t test, Fig. 10G). There was also no significant correlation between the change in strength of inhibition and change in duration of inhibition due to application of STRY (r = -0.1; p > 0.05).

Inhibition at early delays was not eliminated in 14 of 16 units tested with BIC (Table 1). However, the average strength of inhibition was significantly reduced (Control mean: -0.67; BIC mean: -0.51, p < 0.01, paired t test, Fig. 10B). BIC application had different effects on inhibition at early delays across the population. For 9 units in which the delay width of inhibition could be measured, there was no significant reduction in the average inhibitory delay width due to application of BIC (Control mean: 8.8 ms, BIC mean: 7.7, p = 0.17, paired t test, Fig. 10H). There was no significant correlation between the change in strength of inhibition and the change in delay width of inhibition due to application of BIC (r = 0.24; p > 0.05).

After combined application of BIC and STRY, inhibition at early delays persisted in 9 of 11 units (Table 1). However, the average strength of inhibition was significantly reduced, from - 0.65 to -0.33 (p < 0.05, paired t test, Fig. 10C). As with separate BIC and STRY application, BIC+STRY had varied effects on the strength of this inhibition across the sample. For 6 units in which the delay width of inhibition at early delays could be measured, there was no significant reduction due to BIC+STRY (Control: 9.2 ms, BIC+STRY: 4.7 ms, p > 0.05, paired *t* test, Fig. 10I). There was no significant correlation between the change in strength of inhibition and change in the duration of inhibition due to application of BIC and STRY (r = -0.03; p > 0.05).

The results from application of the drugs suggest that the rapid inhibition at early delays depends, in part, on inhibitory mechanisms that are independent of glycine or GABA_A receptors in the IC. Nonetheless, in most units, the action of glycine or GABA_A receptors in the IC may contribute to this inhibition.

Inhibition at later delays. Inhibition at delays later than the best facilitatory delay was tested in 19 units. For the unit shown in Figure 11, application of STRY eliminated the inhibition at later delays (delays between 14-16 ms). Further, addition of BIC did not change the effect of STRY. STRY application eliminated inhibition at later delays in 6 of 9 units tested (Table 1) and reduced the inhibition in the remaining 3 units. Across the sample, the average strength of inhibition was significantly reduced from -0.36 to -0.11 (p < 0.01, paired t test, Fig. 10D).

BIC application was less effective, eliminating the inhibition in only 2 of 10 units (Table 1). For the sample of 10 units tested with BIC, the average strength of inhibition was significantly reduced from -0.32 to -0.20 (p < 0.05, paired *t* test, Fig. 10E). The duration of inhibition at later delays could be measured in 5 of the 8 units for which BIC did not eliminate

inhibition. There was no significant reduction in the width of inhibition at later delays due to application of BIC (Control: 10.8 ± 1.4 ms, BIC: 9.8 ± 2.9 , p = 0.69, paired *t* test).

Combined application of BIC and STRY showed effects similar to STRY application. In 10 of 12 units (Table 1) tested, BIC+STRY application eliminated the inhibition at later delays. Across the sample, the average strength of inhibition was significantly reduced from -0.36 to - 0.09 (p < 0.01, paired *t* test, Fig. 10F). These results suggest that the inhibition at later delays is more dependent on glycinergic inhibition than on GABAergic inhibition.

DISCUSSION

Neurons that respond to combinations of spectral elements implement a general strategy for analysis of vocalizations and other complex sounds. Many of these neurons derive selectivity to combinations of elements in vocal signals based on temporally-dependant response facilitation evoked by inputs with distinct frequency tuning. The response facilitation may be accompanied by inhibitory interactions evoked by one or the other of the spectral elements. This inhibition may contribute mechanistically to response facilitation, or it may modify the response selectivity resulting from the facilitation. For combination-sensitive neurons in the mustached bat's IC, the results show that inhibition acts in both of these roles. Mechanistically, glycinergic input to the IC, tuned to the lower of two frequency bands, is essential for the expression of response facilitation while GABAergic inhibition plays a significantly lesser role. In modifying response selectivity, low frequency inhibition alters the timing (best delay) of facilitatory interactions, but not their selectivity for delay (delay width) or their strength. This role for inhibition appears to originate in neural interactions below the inferior colliculus, since the early inhibition evoked by low frequency signals is not eliminated by blockade of glycine and GABA_A receptors within the

IC. Together, these results further specify a sequence of integrative interactions in the ascending auditory pathway up to the inferior colliculus that creates highly selective responses to complex vocal signals.

Inhibitory interactions in facilitatory combination-sensitive IC neurons

Both BF and low frequency inputs to combination-sensitive neurons may activate excitation and inhibition. Nonetheless, the interactions revealed in the delay sensitivity functions (e.g., Fig. 2) are largely the result of the excitation and inhibition evoked by the lower frequency signal, because in most neurons the response to the higher frequency signal is significantly larger than the response to the lower frequency signal. Three-quarters of the neurons (76%) displayed inhibition in addition to the response facilitation evoked by a low frequency signal. Inhibition at early delays was more common, occurring in 59% of all neurons, while inhibition at later delays occurred in 37% of the neurons. One-fifth of neurons showed inhibition at both early and later delays.

There are some clear relationships between inhibitory properties and facilitation. The strongest relates the inhibition at early delays and the timing of the facilitation. First, as has been reported previously (Olsen and Suga, 1991b; Portfors and Wenstrup, 1999) neurons with inhibition at early delays have on average significantly longer best facilitatory delays than do neurons without inhibition at early delays. Second, there is a strong positive correlation between the duration of the inhibition at early delays and the best facilitatory delay. These results indicate that the temporal positioning of the response peak (in terms of delay) is dependent on the inhibition, as has been observed in neurons sensitive to interaural time differences (ITDs) in gerbil medial superior olive (Brand et al. 2002) and barn owl external nucleus of IC (Zheng and Knudsen 1999). Further, these results suggest that some component of the inhibition at early

delays determines the timing of the low-frequency evoked excitation, a suggestion in accord with models of post-inhibitory rebound used to explain response facilitation (Casseday et al. 1994; Wenstrup and Leroy 2001).

A second clear relationship exists, though not as strong, between the strength and timing of the facilitation and the subsequent inhibition at later delays. As expected, the best longer inhibitory delay is well correlated with the best facilitatory delay, but the results suggest that the inhibition is also influenced by the duration (e.g., delay width) and strength of the facilitatory interaction. The later inhibition, or suppression, could thus be activated by the facilitation within the IC neuron. This possibility is consistent with our observation that the inhibition at later delays has little influence on the best facilitatory delay of these units. It is also consistent with our iontophoretic results showing that strychnine usually eliminated this inhibition. We believe this occurred because strychnine removed the facilitation that may evoke this later inhibition.

Some expected relationships did not materialize. For example, there was no relationship between the strength of inhibition at early delays and any measure of the subsequent facilitation. We had hypothesized that the strength of early inhibition may alter the facilitation, since glycinergic input appears to be an essential component of facilitatory interactions. However, we detected no effect. One possible explanation is that the early inhibition is the result of multiple interactions at different levels of the auditory brainstem and midbrain. The glycinergic inhibition acting on IC neurons may form only one component of this early inhibition, with the result that its correlation with delay-tuned facilitation was obscured.

We also hypothesized that the low frequency inhibition would restrict the duration of delay-tuned facilitation (delay width). This corresponds to a presumed role of lateral inhibition that is characteristic of many frequency tuning curves in the auditory system or the center-

surround organization of retinal ganglion cells and lateral geniculate neurons in the visual system. Although neurons with the longest best delays (\geq 15 ms) all lacked low frequency inhibition at later delays, there was no relationship between the duration of the facilitatory interaction and the presence or absence of inhibition. In terms of encoding properties, these observations suggest that low frequency inhibition does not function to restrict the range of delays to which the neuron responds, but instead acts to enhance the contrast between preferred delays and non-preferred delays. By comparison, inhibition acting on IC neurons in the barn owl that are sensitive to interaural timing differences (ITD) both enhances contrast and sharpens delay selectivity (Fujita and Konishi 1991).

Origins of facilitatory and inhibitory interactions

The present results support the hypothesis that combination-sensitive facilitation originates within the mustached bat's inferior colliculus. Wenstrup et al. (1999) proposed that the facilitatory interactions are created in the IC by the convergence of high and low frequency brainstem inputs. The elimination of facilitatory interactions by strychnine application in this and a previous study (Wenstrup and Leroy, 2001) provide strong support for that proposal. Moreover, there are very few facilitatory interactions in nuclei of the lateral lemniscus (Portfors and Wenstrup, 2001) and none in the cochlear nucleus (Marsh and Wenstrup, 2002), both major sources of input to IC combination-sensitive neurons (Wenstrup et al., 1999). Although combination-sensitive facilitatory interactions were first reported in the auditory cortex of this animal (Suga et al., 1978) and subsequently in the MGB (Olsen and Suga 1991a, 1991b), physiological, anatomical, and pharmacological evidence all support the conclusion that these facilitatory interactions originate in the IC.

In contrast to facilitatory interactions, the inhibition at early delays observed in facilitated combination-sensitive neurons appears to be created at levels below the IC. In nearly all units, application of strychnine, bicuculline, or both together failed to eliminate the inhibition at early delays. These results are consistent with physiological studies showing the presence of combination-sensitive inhibition in both the cochlear nucleus (Marsh and Wenstrup, 2002) and lateral lemniscal nuclei (Portfors and Wenstrup, 2001). In these brainstem neurons, responses to BF sounds are suppressed by simultaneous lower frequency signals, with temporal sensitivity that matches the early inhibition described in this study. We believe that such neurons send excitatory projections to the IC, as described later in the Discussion, and dominate the excitatory response of facilitatory neurons that show early inhibition. However, while the strength of early inhibition in the IC facilitatory neurons was not eliminated, it was significantly reduced in the population by the application of the drugs. There are two possible explanations of this result. First, low-frequency tuned glycinergic and GABAergic input from brainstem nuclei terminate onto the high-frequency tuned neurons of the IC to create a purely inhibitory effect. If true, such inhibitory interactions within the IC repeat the interactions occurring in the auditory brainstem. A second explanation is that high-frequency tuned glycinergic and GABAergic inputs preferentially suppress high-frequency excitatory inputs that do not show combination-sensitive inhibition. Blockade of the inhibitory inputs would result in less inhibition at early delays. Neither possibility can be ruled out.

Like facilitation, inhibition at later delays was eliminated in the majority of neurons tested with STRY (67%), and BIC+STRY (83%). In contrast, BIC eliminated inhibition at later delays in only 20% of the neurons tested. These data suggest that inhibition at later delays originates in the IC and may be related to mechanisms creating the facilitation.

Special contribution of glycinergic input to combination-sensitive facilitation in the IC

The majority of studies addressing the physiological response properties of auditory neurons in the IC show similar roles for glycine and GABA_A receptors. Antagonists of both receptors result in changes in temporal response patterns, evoked discharge rate, duration tuning, and latencies (Burger and Pollak, 1998; Casseday et al., 1994, 2000; Fuzessery et al, 2003; LeBeau et al., 1996, 2001; Vater et al., 1992). In the present study, we also observed similar effects on the BF response when we blocked glycine or GABA_A receptors, particularly with respect to temporal response patterns and discharge rate.

In contrast, this study demonstrates a much more substantial role for glycinergic input in combination-sensitive, facilitatory interactions in the auditory midbrain. In this and a previous study (Wenstrup and Leroy, 2001), blockade of glycine receptors by strychnine ALWAYS eliminated or drastically reduced facilitation. Blocking GABA_A receptors by bicuculline eliminated facilitation in about one-quarter of the neurons tested, and in some of these there were strong indications that the loss of facilitation was due to rate saturation effects. Moreover, in neurons where bicuculline did not eliminate facilitation, the addition of strychnine did. This demonstrates that facilitation in most neurons requires glycinergic input and does not require GABAergic input. The minor role of GABA_A-mediated mechanisms is reinforced by our observations that BIC had little effect on response latency, best facilitatory delay, facilitatory delay width, or strength of facilitation. Such specific effects of glycinergic inputs to the IC have not been reported previously. A subsequent section discusses possible mechanisms for this specificity.

An alternative to the conclusion that glycine contributes to response facilitation in the IC is that the observed effects of strychnine to eliminate facilitation can be explained by its action to

increase neuronal discharge rate. This saturation hypothesis thus considers that the facilitatory interactions are still present during strychnine application, but views the loss of measurable facilitation as resulting from the neuron's inability to increase its discharge rate beyond the response to the BF tone. Although we cannot discount this possibility for each recorded unit, the behavior of the population is not consistent with it. First, the saturation hypothesis would predict that the increase in discharge rate to BF or combination tones would be strongly correlated with the reduction in measurable facilitation. There was no such correlation in any drug application regime. Moreover, application of BIC raised response rates much more than did STRY, but eliminated facilitation in many fewer units. Finally, for all units in which facilitation was removed by STRY or BIC+STRY, the rate-level functions suggested that the units were able to respond at higher discharge rates, but did not. This occurred, we believe, because the facilitationy mechanism was blocked by STRY.

For inhibition at early delays, a differential role of glycinergic and GABAergic mechanisms in the IC was not observed. Both STRY and BIC failed to eliminate inhibition at early delays, but caused a similar reduction in the strength of inhibition. For whatever mechanism underlies this reduction in inhibition (see previous section), the results suggest that glycinergic and GABAergic mechanisms contribute similarly.

Inhibition at later delays appears to be substantially more sensitive to the application of STRY than to BIC. This suggests that inhibition at later delays, like facilitatory interactions, is mediated by glycinergic mechanisms in the IC and may be mechanistically related to facilitation. *Neural mechanisms underlying facilitatory interactions*

Suga and colleagues (Suga et al., 1990; Olsen and Suga, 1991b) proposed that combination-sensitive facilitatory interactions occur when excitations in response to lower and

higher frequency sounds coincide. This coincidence model predicts that the difference in latencies of excitation to the two signals will match the best facilitatory delay, a prediction confirmed in both the IC (Portfors and Wenstrup, 1999) and MGB (Olsen and Suga, 1991b). Since many neurons show facilitatory peaks at positive delays (i.e., when the BF sound follows the low frequency sound), the excitation due to the low frequency signal must be delayed in order to coincide with the excitation due to the BF signal. The delay at which facilitation occurs is a key feature of combination-sensitive neurons, encoding the distance of sonar targets or the timing of spectral elements in social vocalizations. Mechanisms that seek to explain these facilitatory interactions should also explain how a range of low frequency latencies is created. Below we discuss possible mechanisms by which glycinergic input may contribute to these features of delay-tuned facilitation.

Disinhibition hypothesis—glycine is inhibitory. A potential explanation of the experimental results is that both the low and high frequency excitations are created by excitatory neurotransmitters, and that glycinergic input has a purely inhibitory effect within a disinhibitory network. As illustrated in Figure 12A, facilitation is created due to the coincidence of excitations due to high and low frequency inputs on the combination-sensitive neuron. However, the GABAergic inhibition (timed to the low frequency input) suppresses the facilitation at all delays except at the best delay. At the best delay, the glycinergic inhibition (timed to the high frequency input and acting on GABAergic neuron), suppresses the GABAergic inhibition on the combination-sensitive neuron, thus allowing facilitation to be expressed. While the predictions of the model for the application of STRY and BIC agree with experimental data, the model fails to explain the effect of BIC+STRY. We have also considered other disinhibition roone timed to the high

frequency response) by appropriately timed glycinergic inhibition. However, the predictions of these models fail to explain the results observed with the application of BIC. Thus, we believe that facilitatory interactions are unlikely to be created by disinhibition mechanisms.

Presynaptic facilitation hypothesis—glycine enhances glutamate release. In the medial nucleus of the trapezoid body (MNTB), application of glycine onto the large calyceal synapse enhances the excitatory postsynaptic current (EPSC) in MNTB neurons (Turecek and Trussell, 2001). The enhancement of the EPSC is thought to result from a depolarizing effect of glycine on the presynaptic terminal, due to a more positive chloride equilibrium potential, and the depolarization increases the release of glutamate at the synaptic terminal.

A similar mechanism could explain the role of glycine in combination-sensitive facilitation. In the model illustrated in Figure 12B, an appropriately timed, low-frequency tuned glycinergic input, located on the presynaptic terminal of the high frequency input, could cause an increase in glutamate release evoked by the high frequency input. The increase in glutamate release would result in a response facilitation in the postsynaptic neuron that is dependant on the timing of the glycinergic and glutamatergic inputs. The predictions of the model regarding drug application agree with our experimental findings. This model explains why most facilitated neurons show no excitation resulting from low frequency sound by itself. It is also attractive because it does not require that postsynaptic glycine receptors have a different effect from GABA_A receptors, both of which increase chloride conductance. It is not known whether axoaxonic glycine terminals occur in the mustached bat's IC, but none have been observed in the ICC of rats (Liu et al., 1999). In the absence of presynaptic terminals, it is possible that glycine released by postsynaptic terminals could diffuse and bind to presynaptic glycine receptors on the high-frequency glutamatergic input, but the temporal constraints (rapid onset and termination) of

facilitatory interactions may not fit the temporal features of this diffusion process. Thus, given our current understanding of the anatomical substrate and temporal requirements of the presynaptic facilitation hypothesis, it appears unlikely to contribute to combination-sensitive facilitatory interactions in the ICC.

Postinhibitory rebound facilitation hypothesis—glycinergic inhibition activates rebound excitation. This hypothesis proposes that an inhibitory glycinergic input, tuned to the low frequency signal, activates a postinhibitory rebound excitation (Fig.13). When the rebound excitation due the low frequency signal coincides with the excitation due to the BF signal, the neuronal discharge rate is maximum. We favor this model because it successfully predicts the results of BIC and STRY application, because there are several possible mechanisms underlying it, and because it can explain some of the temporal features of combination-sensitive facilitation. Below are described several aspects of the model, both positive features and lingering questions.

Two mechanistic issues to be addressed concern how the rebound occurs and how the facilitation occurs. There are several mechanisms that could explain the post-inhibitory rebound excitation. These include M currents (Barrio et al., 1994), I_h currents (Koch and Grothe 2003), low-threshold Ca²⁺ currents (Tell and Bradley 1994), Ca²⁺-activated Cl⁻ currents (Owen et al., 1984), inward rectifying currents (Chandler et al, 1994), and anode break excitation (Hodgkin and Huxley, 1952). These could generate either a membrane depolarization or an increased membrane excitability following glycine-mediated opening of chloride channels. In the first of these possibilities, the rebound excitation sums with the high frequency evokes excitation to cause response facilitation. In the second possibility, a change in the postsynaptic membrane, perhaps in the input resistance of the neuron, causes the cell to respond more strongly to the high frequency input. Intracellular recordings may help to distinguish these possibilities.

An expectation of the postinhibitory rebound hypothesis is that the neuron is less excitable during a period preceding the rebound excitation. In facilitated combination-sensitive neurons, this would be revealed by a suppression of high frequency-evoked spikes when these occur prior to the low frequency-evoked excitation, corresponding to "early inhibition" (Figs. 1,2). Early inhibition was observed in about 60% of facilitated units. While much of this early inhibition was insensitive to BIC or STRY application, suggesting an origin below the IC, the component of early inhibition that was removed by STRY may be an observable feature of the postinhibitory rebound mechanism. However, in 40% of facilitated neurons we found no evidence of early inhibition in the delay sensitivity curves. One explanation is that the inhibition was too short to be observed by delay curves for which the resolution was 2 ms. This explanation is plausible because these neurons lacking observable early inhibition had short best facilitatory delays. As discussed below, these require very short periods of early inhibition. A second explanation is that the glycinergic input evokes weak inhibition that is unable to suppress the high frequency-evoked response, but is still sufficient to activate a rebound facilitation. Consequently, the delay curve would show little or no early inhibition. This explanation is consistent with a quantitative model of postinhibitory rebound and delay sensitivity based on Hodgkin-Huxley equations (Nataraj 2003).

The postinhibitory rebound hypothesis provides a mechanism by which the observed range of best facilitatory delays (-3 to 30 ms) is created. Previous work (Portfors and Wenstrup 1999, Olsen and Suga 1991b) and our data (not shown) indicate that differences in the best facilitatory delay among neurons are largely the result of differences in the latency of the low frequency-evoked excitation. In the population recorded here, the range of low frequency excitatory latencies required is approximately 7–35 ms. Low frequency-tuned glycinergic inputs

to combination-sensitive IC neurons are limited to only a few auditory brainstem regions, the cochlear and lateral lemniscal nuclei (Kemmer and Vater, 1997; Wenstrup et al., 1999; Winer et al., 1995). Among these, the range of latencies cannot account for long-latency low frequency responses. For instance, the maximum observable latency among lateral lemniscal units is 22 ms (Portfors and Wenstrup, 2001). However, if the excitation is created by preceding inhibition, the latency of the low frequency rebound excitation could be extended for several milliseconds or even tens of milliseconds beyond the latency of the input. The variation in best facilitatory delay among neurons may thus result from differences in the duration of the inhibitory period preceding rebound excitation to the low frequency signal. This possibility is supported by invitro studies showing that rebound excitation is delayed by increasing the duration of the preceding inhibition (Bertrand and Cazalets, 1998; Koch and Grothe, 2003). The I_h current can evoke a rebound excitation when the duration of the preceding inhibition is as short as 6 ms (Koch and Grothe, 2003), while low threshold Ca^{2+} activated currents and inward rectifying currents require the preceding inhibition to be at least 60 ms in duration (Bertrand and Cazalets, 1998). The timing of rebound proposed here is more consistent with I_h currents, although even these may be too long for low-frequency excitation among neurons with the shortest best delays.

Both glycine and GABA-A receptors increase chloride conductance in postsynaptic cells. How does this model explain the differential role of glycine and GABA in facilitatory interactions? The simplest explanation is that the low frequency-tuned inputs that activate postinhibitory rebound are glycinergic. The most likely sources of such inputs are the anteroventral cochlear nucleus and the ventral and intermediate nuclei of the lateral lemniscus (Kemmer and Vater. 1997; Wenstrup et al. 1999; Winer et al. 1995). Some of these brainstem

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neurons may also contain GABA (Winer et al. 1995), which could explain why facilitated interactions were blocked by bicuculline in about one-quarter of the neurons.

It seems necessary, however, to explain why most GABAergic inputs do not evoke facilitation and also why high frequency-tuned glycinergic inhibition does not appear to evoke facilitation. One explanation is that there is a spatial segregation of the low frequency glycinergic inputs on the postsynaptic cell, away from either low frequency GABAergic inputs or high frequency-tuned glycinergic inputs. This segregation of inputs, matched to a local population of channel proteins mediating I_h or other currents, might restrict the postinhibitory rebound to the low frequency glycinergic input. While there are no studies directly addressing the spatial distribution of GABA and glycine receptors on individual neurons in the IC, Bartlett et al. (2000) have shown that GABA terminals are found more on the secondary and tertiary dendrites in individual cells in the MGB. Further, there is evidence that glycine and GABA terminations are distributed differently across the mustached bat's IC (Winer et al., 1995). The effect of distribution of receptors on the response of the neurons can be substantial; Andrasfalvy and Magee (2001) show a doubling of AMPA currents with distance from the soma. They suggest that this effect is due to increase in receptor number or variation in receptor density.

In summary, this postinhibitory rebound model suggests that low frequency-tuned glycinergic inputs, terminating on high frequency-tuned neurons of the IC, create a rebound excitation. The timing of the rebound is dependant both on the latency of the glycinergic input and the properties of the postsynaptic neuron that control the duration of the inhibitory period. The coincidence of this excitation with the high-frequency tuned excitatory input evokes the facilitatory response that is key to the selective response of the neuron to the temporal pattern of spectrally distinct inputs.

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Functional implications

In the mustached bat's auditory cortex, several types of combination-sensitive neurons show selective responses to combinations of distinct elements in sonar calls and echoes (O'Neill and Suga 1979; Suga et al. 1978, 1979), or to particular social vocalizations (Esser et al., 1997; Ohlemiller et al., 1996). These selective responses are based on both facilitatory and inhibitory interactions. The present study clarifies the site of origin for several of these interactions, summarized in Figure 14. The inhibition occurring when two spectral elements are presented simultaneously, what we have termed "early inhibition", imposes restrictions on the types and timing of elements in complex sounds to which a neuron can respond. Much of this early inhibition appears to originate below the auditory midbrain, probably in the intermediate nucleus of the lateral lemniscus (Portfors and Wenstrup, 2001). We propose that IC neurons displaying early inhibition receive excitatory input from lateral lemniscal neurons that also display this response property. This input carries the main excitatory response to the BF sound. When that excitation is combined with a low frequency-tuned glycinergic input at the IC neuron, it displays both early inhibition and facilitation to a low frequency signal (Fig. 14). Later inhibition, not shown in Figure 14, appears to arise as a consequence of the facilitatory interaction in the IC. However, there is evidence that higher centers add to this later inhibition, since it is increasingly more common in the MGB (Olsen and Suga, 1991b) and some areas of auditory cortex (Edamatsu and Suga 1993) than among the neurons in this study. This suggests that there are multiple spectral integration events distributed throughout the ascending pathway to auditory cortex. Unlike the visual system, in which integration across the sensory surface seems to occur mostly at cortical levels, much of the integration performed in creating combination-sensitive responses is completed prior to cortical processing.

The majority of neurons recorded in this study are probably involved in the analysis of target distance during echolocation, corresponding to "FM-FM" or delay-tuned neurons described in previous studies (Olsen and Suga, 1991b; Suga et al. 1979). Are these facilitatory interactions in the auditory midbrain specific to the analysis of sonar echoes, or do they participate in analyses of other complex acoustic signals? Among auditory cortical neurons, the combination-sensitive response properties of some FM-FM neurons appear to create selectivity for particular social vocalizations (Esser et al., 1997; Ohlemiller et al., 1996). In the present study, some neurons were facilitated by spectral combinations not used in sonar, and the facilitation was blocked by strychnine. Preliminary evidence suggests that these "non-sonar neurons" can display selective responses to social vocalizations (Portfors et al., 2002). These observations are in agreement with studies in other vertebrate species that relate combinatorial responses to selectivity for social vocalizations (Fuzessery and Feng, 1983; Margoliash and Fortune, 1992; Rauschecker et al., 1995). Although the temporal scale of the integrative interactions may differ, from milliseconds in bat echolocation, to seconds in the case of birdsong, we believe a similar sequence of integrative interactions may underlie the analyses of these complex vocal signals.

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REFERENCES

Adams JC and Mugnaini E. Dorsal nucleus of the lateral lemniscus: a nucleus of GABAergic projection neurons. *Brain Res Bull* 13(4): 585-90, 1984.

Adams JC and Mugnaini E. Immunocytochemical evidence for inhibitory and disinhibitory circuits in the superior olive. *Hear Res* 49(1-3): 281-98, 1990.

Andrasfalvy BK and Magee JC. Distance-dependent increase in AMPA receptor number in the dendrites of adult hippocampal CA1 pyramidal neurons. *J Neurosci* 21: 9151-9159, 2001.

Backoff PM, Palombi PS and Caspary DM. Gamma-aminobutyric acidergic and glycinergic inputs shape coding of amplitude modulation in the chinchilla cochlear nucleus. *Hear Res* 134: 77-88, 1999.

Barrio LC, Araque A and Buno W. Participation of voltage-gated conductances on the response succeeding inhibitory synaptic potentials in the crayfish slowly adapting stretch receptor neuron. *J Neurophysiol* 72(3):1140-51, 1994.

Bartlett EL, Stark JM, Guillery RW and Smith PH. Comparison of the fine structure of cortical and collicular terminals in the rat medial geniculate body. *Neurosci* 100: 811-828, 2000.

Bertrand S and Cazalets JR. Postinhibitory rebound during locomotor-like activity in neonatal rat motoneurons in vitro. *J Neurophysiol* 79: 342-351, 1998.

Brand A, Behrend O, Marquardt T, McAlpine D and Grothe B. Precise inhibition is essential for microsecond interaural time difference coding. *Nature* 417: 543-547, 2002.

Burger RM and Pollak GD. Analysis of the role of inhibition in shaping responses to sinusoidally amplitude-modulated signals in the inferior colliculus. *J Neurophysiol* 80: 1686-1701, 1998.

Caspary DM, Havey DC and Faingold CL. Effects of microiontophoretically applied glycine and GABA on neuronal response patterns in the cochlear nuclei. *Brain Res* 172: 179-185, 1979.

Casseday JH, Ehrlich D and Covey E. Neural tuning for sound duration: role of inhibitory mechanisms in the inferior colliculus. *Science* 264: 847-850, 1994.

Casseday JH, Ehrlich D and Covey E. Neural measurement of sound duration: control by excitatory-inhibitory interactions in the inferior colliculus. *J Neurophysiol* 84: 1475-1487, 2000.

Chandler SH, Hsaio CF, Inoue T and Goldberg LJ. Electrophysiological properties of guinea pig trigeminal motoneurons recorded in vitro. *J Neurophysiol* 71(1):129-45, 1994.

Chen QC and Jen PH. Bicuculline application affects discharge patterns, rate-intensity functions, and frequency tuning characteristics of bat auditory cortical neurons. *Hear Res* 150: 161-174, 2000.

Edamatsu H and Suga N. Differences in response properties of neurons between two delaytuned areas in the auditory cortex of the mustached bat. *J Neurophysiol* 69: 1700-1712, 1993.

Esser KH, Condon CJ, Suga N and Kanwal JS. Syntax processing by auditory cortical neurons in the FM-FM area of the mustached bat Pteronotus parnellii. *Proc Natl Acad Sci* 94: 14019-14024, 1997.

Faingold CL, Boersma Anderson CA and Caspary DM. Involvement of GABA in acoustically-evoked inhibition in inferior colliculus neurons. *Hear Res* Mar;52(1): 201-16, 1991.

Fitzpatrick DC, Suga N and Olsen JF. Distribution of response types across entire hemispheres of the mustached bat's auditory cortex. *J Comp Neurol* 391(3): 353-65, 1998.

Foote SL, Freedman R and Oliver AP. Effects of putative neurotransmitters on neuronal activity in monkey auditory cortex. *Brain Res* 86(2): 229-42, 1975.

Fujita I and Konishi M. The role of GABAergic inhibition in processing of interaural time difference in the owl's auditory system. *J Neurosci* 11(3): 722-39, 1991.

Fuzessery ZM and Feng AS. Mating call selectivity in the thalamus and midbrain of the leopard frog (Rana p. pipens); Single and multiunit Analyses. *J Comp Physiol* [A] 150: 333-344, 1983.

Fuzessery ZM and Hall JC. Role of GABA in shaping frequency tuning and creating FM sweep selectivity in the inferior colliculus. *J Neurophysiol* 76: 1059-1073, 1996.

Fuzessery ZM, Wenstrup JJ, Hall JC and Leroy S. Inhibition has little effect on response latencies in the inferior colliculus. *J Assoc Res Otolaryngol* 4: 60-73, 2003.

Havey DC and Caspary DM. A simple technique for constructing 'piggy-back' multibarrel microelectrodes. *Electroencephal Clin Neurophysiol* 48: 249-251, 1980.

Hodgkin AL and Huxley AF. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* 117(4):500-44, 1952.

Kemmer M and Vater M. The distribution of GABA and glycine immunostaining in the cochlear nucleus of the mustached bat (Pteronotus parnellii). *Cell Tissue Res* 287(3):487-506, 1997.

Koch U and Grothe B. Hyperpolarization-activated current (I_h) in the inferior colliculus: distribution and contribution to temporal processing. *J Neurophysiol* 90(6):3679-87, 2003.

LeBeau FE, Rees A and Malmierca MS. Contribution of GABA- and glycine-mediated inhibition to the monaural temporal response properties of neurons in the inferior colliculus. *J Neurophysiol* 75: 902-919, 1996.

LeBeau FE, Malmierca MS and Rees A. Iontophoresis in vivo demonstrates a key role for GABA(A) and glycinergic inhibition in shaping frequency response areas in the inferior colliculus of guinea pig. *J Neurosci* 21: 7303-7312, 2001.

Liu Y, Sakurai H, Kurokawa K, Yamada H and Kudo M. Glycine-immunoreactive synapses in the central nucleus of the inferior colliculus. An electron microscopic study in the cat. *Neurosci Lett* 269: 183-185, 1999.

Margoliash D and Fortune ES. Temporal and harmonic combination-sensitive neurons in the zebra finch's HVc. *J Neurosci* 12: 4309-4326, 1992.

Marsh RA and Wenstrup JJ. Responses to combination of tones in the cochlear nucelus of awake mustached bats. . *Abstr Assoc Res Otolaryngol* : 2002.

Mittmann DH and Wenstrup JJ. Combination-sensitive neurons in the inferior colliculus. *Hear Res* 90: 185-191, 1995.

Nataraj K. *Neural mechanisms in analyzing complex sounds (PhD dissertation).* Akron, OH: The University of Akron, 2003.

O'Neill WE and Suga N. Target range-sensitive neurons in the auditory cortex of the mustache bat. *Science* 203(4375):69-73, 1979.

Ohlemiller KK, Kanwal JS and Suga N. Facilitative responses to species-specific calls in cortical FM-FM neurons of the mustached bat. *Neuroreport* 7: 1749-1755, 1996.

Olsen JF and Suga N. Combination-sensitive neurons in the medial geniculate body of the mustached bat: encoding of relative velocity information. *J Neurophysiol* 65: 1254-1274, 1991a.

Olsen JF and Suga N. Combination-sensitive neurons in the medial geniculate body of the mustached bat: encoding of target range information. *J Neurophysiol* 65: 1275-1296, 1991b.

Owen DG, Segal M and Barker JL. A Ca-dependent Cl- conductance in cultured mouse spinal neurones. *Nature* 311(5986):567-70, 1984.

Park TJ and Pollak GD. GABA shapes sensitivity to interaural intensity disparities in the mustache bat's inferior colliculus: implications for encoding sound location. *J Neurosci* 13(5):2050-67, 1993.

Portfors CV, Gans DP and Wenstrup JJ. Responses to communication calls in the inferior colliculus of the mustached bat. *Abstr Assoc Res Otolaryngol* : 2002.

Portfors CV and Wenstrup JJ. Delay-tuned neurons in the inferior colliculus of the mustached bat: implications for analyses of target distance. *J Neurophysiol* 82: 1326-1338, 1999.

Portfors CV and Wenstrup JJ. Responses to combinations of tones in the nuclei of the lateral lemniscus. *J Assoc Res Otolaryngol* 2: 104-117, 2001.

Rauschecker JP, Tian B and Hauser M. Processing of complex sounds in the macaque nonprimary auditory cortex. *Science* 268: 111-114, 1995.

Saint Marie RL, Ostapoff EM, Morest DK and Wenthold RJ. Glycine-immunoreactive projection of the cat lateral superior olive: possible role in midbrain ear dominance. *J Comp Neurol* 279(3): 382-96, 1989.

Suga N, O'Neill WE, Kujirai K and Manabe T. Specificity of combination-sensitive neurons for processing of complex biosonar signals in auditory cortex of the mustached bat. *J Neurophysiol* 49: 1573-1626, 1983.

Suga N, O'Neill WE and Manabe T. Cortical neurons sensitive to combinations of information-bearing elements of biosonar signals in the mustache bat. *Science* 200(4343):778-81, 1978.

Suga N, O'Neill WE and Manabe T. Harmonic-sensitive neurons in the auditory cortex of the mustache bat. *Science* 203(4377): 270-4, 1979.

Suga N, Olsen JF and Butman JA. Specialized subsystems for processing biologically important complex sounds: cross-correlation analysis for ranging in the bat's brain. *Cold Spring Harbor symposia on quantitative biology* 55: 585-597, 1990.

Tell F and Bradley RM. Whole-cell analysis of ionic currents underlying the firing pattern of neurons in the gustatory zone of the nucleus tractus solitarii. *J Neurophysiol* 71(2):479-92, 1994.

Turecek R and Trussell LO. Presynaptic glycine receptors enhance transmitter release at a mammalian central synapse. *Nature* 411: 587-590, 2001.

Vater M, Habbicht H, Kossl M and Grothe B. The functional role of GABA and glycine in monaural and binaural processing in the inferior colliculus of horseshoe bats. *J Comp Physiol [A]* 171: 541-553, 1992.

Wang J, Caspary D and Salvi RJ. GABA-A antagonist causes dramatic expansion of tuning in primary auditory cortex. *Neuroreport* 11: 1137-1140, 2000.

Wenstrup JJ and Leroy SA. Spectral integration in the inferior colliculus: role of glycinergic inhibition in response facilitation. *J Neurosci* 21: RC124 (1-6), 2001.

Wenstrup JJ, Mittmann DH and Grose CD. Inputs to combination-sensitive neurons of the inferior colliculus. *J Comp Neurol* 409: 509-528, 1999.

Winer JA, Larue DT and Pollak GD. GABA and glycine in the central auditory system of the mustache bat: structural substrates for inhibitory neuronal organization. *J Comp Neurol* 355(3): 317-53, 1995.

Winer JA, Saint Marie RL, Larue DT and Oliver DL. Gabaergic feedforward projections from the inferior colliculus to the medial geniculate body. *Proc Natl Acad Sci* 93: 8005-10, 1996.

Yang L, Pollak GD and Resler C. GABAergic circuits sharpen tuning curves and modify response properties in the mustache bat inferior colliculus. *J Neurophysiol* 68: 1760-1774, 1992.

Zheng W and Knudsen EI. Functional selection of adaptive auditory space map by GABA_A-

mediated inhibition. Science 284(5416): 962-5, 1999.

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FIGURE LEGENDS

Fig. 1. Response as function of delay between two tones of different frequency reveals inhibitory and facilitatory interactions. Facilitation, inhibition at early delays, and inhibition at later delays each can be described by three quantitative measures: best delay of interaction, range of delays over which the interaction occurred (delay width), and strength of interaction measured at best delay of interaction. A: The response of a combination-sensitive neuron as a function of delay is expressed as spike counts. The BF of the unit was 81.6 kHz and the interacting low frequency sound was tuned to 23.6 kHz. B: Delay function replotted to show the strength of the interaction as a function of delay. The *two horizontal dashed lines* represent the threshold for facilitation (0.09) and inhibition (-0.11). *Vertical dashed lines* show delay width (*DW*) of interactions for facilitation (6.2 ms), inhibition at early delays (6.5 ms), and inhibition at later delays (7.3 ms).

Fig. 2. Types of facilitated combination-sensitive units based on inhibitory interactions. A: The best facilitatory interaction for this unit was at 0 ms delay, with no inhibition at early or later delays. B: This unit has its best facilitatory interaction at 12 ms delay, with inhibition only at early delays (0-4 ms). C: For this unit, the best facilitatory interaction was at 2 ms delay, with inhibition only at later delays (8-10 ms). D: This unit had its best facilitatory interaction at 4 ms delay with inhibition at early delays (-2–0 ms) and at later delays (12–16 ms). For each unit, the responses of the unit to individual tones and combination of tones are displayed as delay sensitivity functions and peristimulus time histograms (PSTHs). In delay sensitivity functions, response to individual tones is shown to the right of the delay function. In PSTHs, the timing and duration of the BF and low frequency tones are shown by *filled and unfilled rectangles*, respectively. *Numbers* to the right of each PSTH indicate the spike count. *Dotted vertical lines* in

some PSTHs indicate the time window within which response magnitude was evaluated. In these cases, spike counts and delay curves are based on time-windowed responses.

Fig. 3. Distribution of facilitated combination-sensitive units based on the presence and timing of inhibition.

Fig. 4. Distribution of features of combination-sensitive interactions (best delay, delay width, strength of interaction) for facilitation (A), inhibition at early delays (B) and inhibition at later delays (C).

Fig. 5. Relationships between different features of facilitation and inhibition. A,B: best facilitatory delay was significantly correlated with the best short inhibitory delay (A) and width of inhibition at early delays (B). C,D: delay width of inhibition at later delays was significantly correlated with delay width of facilitation (C) and with strength of facilitation (D). *Asterisks* indicate significance at p < 0.001.

Fig. 6. Facilitatory combination-sensitive interactions in the mustached bat's IC are eliminated by application of strychnine (STRY). A: Response magnitude of a facilitated unit as a function of delay between low and high frequency signals before (Control) and during application of STRY (20 nA). For this unit, application of STRY eliminated the facilitatory peak at 10 ms delay (Interaction Index: Control, 0.16; STRY, 0.01) and had little effect on the strength of inhibition at 0 ms delay (Interaction Index: Control, -0.65; STRY, -0.63). B: STRY eliminated facilitation in a unit that showed interactions between frequency combinations not used in sonar signals. The low frequency signal was tuned to frequencies outside of the sonar call. For this unit, application of STRY elevated the response to the high frequency signal and eliminated the facilitatory peak at 0 ms delay. See Figure 2 for details of delay sensitivity function and PSTHs.

Fig. 7. Facilitatory combination-sensitive interactions in the mustached bat's IC were not eliminated by application of bicuculline (BIC). A: Response magnitude of a facilitated unit as a function of delay between low and high frequency signals under three conditions: control, BIC application (15 nA), and BIC combined with STRY (15 nA) (BIC+STRY). For this unit, application of BIC elevated the response magnitude but did not eliminate the facilitatory peak at 6 ms delay (Interaction Index: Control, 0.2; BIC, 0.39; BIC+STRY, -0.09) or the inhibition at 0 ms delay (Interaction Index: Control, -0.59; BIC, -0.71; BIC+STRY, -0.36). Addition of STRY further elevated the response magnitude, eliminate the facilitation in a unit that showed interactions between frequency combinations not occurring in sonar signals. The low frequency signal was tuned to frequencies outside of the sonar call. Application of BIC (20 nA) substantially raised this unit's discharge rate but did not eliminate facilitation. See Figure 2 for details.

Fig. 8. Quantitative effects of drugs on facilitatory interactions and response magnitudes. A-C: Strength of facilitation before and during application of STRY (A), BIC (B), and BIC+STRY (C). Application of STRY or BIC+STRY eliminated or substantially reduced facilitation in all cases, while BIC eliminated facilitation in many fewer units. Dashed lines indicate index values corresponding to thresholds for facilitation (0.09) and inhibition (-0.11). The *separate dots and error bars* indicate the mean and the standard error of the mean for the sample. *Asterisks* indicate significant differences between the control and drug tests using paired *t*-tests (* p < 0.05, ** p < 0.01). D-F: Response magnitude to combination tones at the best facilitatory delay before and during application of STRY (D), BIC (E), and BIC+STRY (F). For each group, drug application caused a significant increase in the response magnitude. G-I:

Response magnitude to high frequency tone before and during application of STRY(G), BIC (H), and BIC+STRY (I). For each group, drug application caused a significant increase in the response magnitude. J-L: Response magnitude to low frequency tone before and during application of STRY (J), BIC (K), and BIC+STRY (L). Drug application failed to significantly alter the low frequency response in any group.

Fig. 9. BIC had little effect on the best delay of facilitation and a varied effect on the width of facilitation. A: The best delay of facilitation was not generally effected by application of BIC (Control: 5.9 ± 1.1 ms, BIC: 5.9 ± 1.1 ms, p > 0.1, paired *t* test). B: There was no significant change in the width of facilitation (Control: 6.9 ± 0.92 ms, BIC: 6.9 ± 0.81 ms, *p* > 0.1, paired *t* test). In this population, approximately half showed a reduction in width of facilitation and others showed increase in width of facilitation. See Figure 8 for details.

Fig. 10. Inhibition at early delays (A-C) is reduced but rarely eliminated by application of STRY, BIC, or BIC+STRY. Inhibition at later delays (D-F) is usually eliminated by application of STRY or BIC+STRY, but not by BIC. Width of inhibition at early delays (G-I) is sometimes reduced by application of drugs. See Figure 8 for details.

Fig. 11. Both facilitation and inhibition at later delays is eliminated by application of STRY or BIC+STRY. A: Response magnitude of a facilitated unit as a function of delay between low and high frequency signals under three conditions: control, STRY application (20 nA), and BIC+STRY (BIC, 25 nA). B: Response magnitude in the delay sensitivity function shown in A is re-plotted as interaction index values (see Methods). Values of 0.09 (*upper dashed line*) or higher indicate facilitatory interactions. Values of -0.11 (*lower dashed line*) or below indicate inhibitory interactions. For this unit, application of STRY elevated the response magnitude,

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eliminated the facilitatory peak at 6 ms delay and the inhibition at 14 ms delay, and reduced the inhibition at 0 ms delay. Application of BIC+STRY had little additional effect.

Fig. 12. Two models to explain facilitated combination-sensitive responses, with comparisons between predicted and experimental results of drug application. A. In this disinhibition model, facilitation is due to the coincidence of excitatory inputs responding to high and low frequency sounds. The facilitation is inhibited by an appropriately timed GABAergic low frequency input. At the best delay, the GABAergic neuron. This allows facilitation to be expressed at some delays. This model does not predict the observed results of drug application. B. In this presynaptic facilitation model, glycine released by the low frequency input acts on glycinergic receptors located on the presynaptic terminal of a high frequency tuned input. Under favorable chloride concentration ratios, the glycine increases glutamate release. Facilitation will occur as the low frequency-evoked glycine release coincides with high frequency-evoked glutamate release. The text describes limitations of this model. +: excitation, -: inhibition, *GluR*: glutamate receptor, *GlyR*: glycine receptor, *CS*: combination-sensitive.

Fig. 13. Postinhibitory rebound model of facilitation. Facilitation is due to the coincidence of excitation to high frequency sound and a rebound excitation to the low frequency sound. The predictions of the model agree with experimental data. *Elim*: elimination, *No Elim*: No Elimination.

Fig. 14. The combination-sensitive response properties of IC neurons may arise through a sequence of integration interactions in the auditory brainstem and midbrain. The delay-sensitive response of a facilitatory neuron in IC (*right*) displays early inhibition, facilitation, and later inhibition. Such neurons in the IC receive two major inputs: a low frequency-tuned glycinergic

input (*filled arrow*) that evokes a rebound excitation causing facilitation, and an excitatory input from a brainstem neuron that shows combination-sensitive inhibition (*hatched arrow*). The lower brainstem combination-sensitive neuron integrates two inputs: an excitatory high frequency input and an inhibitory low frequency input that suppresses the high frequency response when presented simultaneously. See Figure 12 for abbreviations.

Table 1

Table 1. Effect of strychnine (STRY), bicuculline (BIC), and BIC+STRY on facilitation, inhibition at early delays, and inhibition at later delays.

		STRY	BIC	BIC+STRY
		N (%)	N (%)	N (%)
Faciliation	Eliminated	22 (96)	7 (28)	20 (100)
	Not Eliminated	1(4)	18(72)	
Inhibition at early delays	Eliminated	0 (0)	2 (12)	2 (18)
	Not Eliminated	15 (100)	14 (88)	9 (82)
Inhibition at later delays	Eliminated	7 (78)	2 (20)	10 (83)
	Not Eliminated	2 (22)	8 (80)	2 (17)

<u>Fig 1</u>



Fig 2



<u>Fig 3</u>



<u>Fig 4</u>



<u>Fig 5</u>



<u>Fig 6</u>



<u>Fig 7</u>



<u>Fig 8</u>



<u>Fig 9</u>



<u>Fig 10</u>







<u>Fig 12</u>



<u>Fig 13</u>



Fig 14

