Glycinergic Inhibition Creates a Form of Auditory Spectral Integration in Nuclei of the Lateral Lemniscus

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Submitted 14 January 2009; accepted in final form 2 June 2009


INTRODUCTION

Our understanding of information processing in ascending auditory pathways is shaped by the view that individual neurons respond to a limited range of sound frequencies and that different groups of neurons represent different frequencies of sound. Although this seems to be true to a first approximation, in fact many auditory neurons respond to a broader frequency range based on forms of spectral integration. This begins in the cochlea, where hair cells and auditory nerve (AN) fibers respond to low-frequency (LF) signals in addition to the higher frequencies to which they are more sensitive (Russell and Sellick 1978; Sachs and Kiang 1968). As early as the dorsal cochlear nucleus (DCN), neuronal responses are influenced by a wideband inhibitor that projects onto frequency-tuned DCN neurons (Nelken and Young 1994). In the auditory forebrain, a variety of forms of spectral integration include multipeaked tuning, broad tuning, and a variety of nonlinear interactions in response to distinct spectral inputs (Abeles and Goldstein Jr 1972; Fuzessery and Feng 1983; Kadia and Wang 2003; Rauschecker et al. 1995; Shamma and Symmes 1985; Suga et al. 1979; Sutter and Schreiner 1991). The central mechanisms that underlie these spectrally integrative responses are in most cases not understood. The present study provides evidence that types of spectral integration in which LF sounds suppress a neuron’s response to its best (characteristic) frequency originate in the early stages of the ascending auditory pathway.

The types of spectral integration studied here are characterized by suppression of responses to sounds near a neuron’s best frequency (BF) by sounds at least an octave lower in frequency. Such neurons are common throughout the mustached bat’s auditory midbrain (Mittmann and Wenstrup 1995; Nataraj and Wenstrup 2006; O’Neill 1985; Portfors and Wenstrup 1999), thalamus (Wenstrup 1999), and cortex (Kanwal et al. 1999). They also occur in other species and/or auditory centers (Imig et al. 1997; Kadia and Wang 2003; Portfors and Felix 2nd 2005; Rauschecker et al. 1995; Shamma and Symmes 1985; Sutter et al. 1999). These response properties serve to limit the responsiveness of neurons to complex spectra with energy at the lower, suppressive frequencies.

Low-frequency suppression of high-frequency responses occurs in the majority of mustached bat IC neurons. There appear to be two major classes of LF suppression, distinguished in part by frequency tuning and associated excitatory responses. One class is generally tuned to frequencies <23 kHz and is independent of the neuron’s BF. For this class of responses, some low-frequency excitation may occur, always preceding or occurring simultaneously with the suppression of high-frequency responses. We have speculated that this LF suppression is cochlear in origin (Marsh et al. 2006; Nataraj and Wenstrup 2006). A second class of suppression is best activated by sound energy in the 23- to 30-kHz range associated with the fundamental of the biosonar signal. This class may or may not be associated with an excitatory response to the 23- to 30-kHz band, but excitation that occurs typically follows the suppression. We have speculated that this suppression is not of cochlear origin (Gans et al. 2009; Nataraj and Wenstrup 2006). For either class, however, blockade of the GABAAergic and/or glycinergic inputs in the inferior colliculus (IC) generally fails to eliminate the LF suppression. This suggests that LF suppression originates below the IC (Nataraj and Wenstrup 2006).

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Since we observed suppression in nuclei of the lateral lemniscus (NLL) (Portfors and Wenstrup 2001), we hypothesized that neural interactions underlying suppression may originate there. The present study tests this hypothesis by blocking GABAAergic and glycinergic inhibition in NLL neurons. The results indicate that the second class of LF suppression, tuned to 23–30 kHz, arises in the ventral and intermediate nuclei of the lateral lemniscus through glycinergic inhibition. This work thus identifies a contribution of processing within NLL to higher-order auditory responses in the auditory midbrain and forebrain.

METHODS

We examined auditory responses in the NLL of seven awake mustached bats (Pteronotus parnellii), captured in Trinidad and Tobago. All procedures used in this study were approved by the Institutional Animal Care and Use Committee of the Northeastern Ohio Universities College of Medicine and follow guidelines set by the National Institutes of Health for the care and use of laboratory animals.

Surgical procedures

Each bat was sedated with butorphanol (5 mg/kg, administered intraperitoneally [ip]; Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA), anesthetized with isoflurane (1.5–2.0%; Abbott Laboratories, North Chicago, IL), and placed in a stereotaxic holder. Depilatory lotion was used to remove hair on the skin overlying the skull. The skin on the head was then disinfected with betadine. To expose the dorsal surface of the skull, a midline incision was made in the skin and the underlying muscles were reflected laterally. A metal pin was cemented onto the rostral portion of the skull to secure the head during physiological experiments. A tungsten wire, cemented into the skull overlying the cerebral cortex, served as ground for electrophysiological recordings. For access to the lateral lemniscal nuclei, surface features and stereotaxic coordinates guided the placement of a small hole in the skull (diameter, <1.0 mm) over the caudal IC, which lies on the dorsal surface of the brain. After surgery, lidocaine (a local anesthetic) was applied to the surgical area and the bat was returned to the holding cage. The bat was allowed to recover for 2–3 days before physiological experiments began.

Acoustic stimulation

Single-tone bursts (11-ms duration, 0.5-ms rise/fall time, four presentations/s) and combinations of tone bursts were computer synthesized, downloaded to a digital signal processor (AP2 Multi-Processor DSP card; Tucker-Davis Technologies [TDT], Alachua, FL), converted to analog signals at a sampling rate of 500 kHz (model DA3-2; TDT), filtered (model FT6-2; TDT), attenuated (model PA4; TDT), summed (model SM3; TDT), and amplified (Parasound model HCA-800II). The signal was sent to a loudspeaker (Infinity EMIT-B tweeter; Harmon International Industries, Woodbury, NY) that was placed 10 cm from the animal and 25° into the sound field contralateral to the NLL under study. The performance of the acoustic system speakers was tested with a single-walled Industrial Acoustics chamber lined with polyurethane foam to reduce echoes. On experimental days, the animal was lightly sedated (butorphanol, 2.5 mg/kg, administered subcutaneously) prior to placement in a stereotaxic apparatus inside the heated acoustic chamber. If at any time the animal showed signs of discomfort or distress, it was removed from the stereotaxic apparatus. Recordings began ≥1 h after sedation. The animal was offered water from a medicine dropper between electrode penetrations. The recording sessions did not exceed 6 h and were limited to one recording session per day.

Physiological activity was amplified, band-pass filtered (600–6000 Hz), and sent through a spike signal enhancer (model 40-46-1; FHC, Bowdoin, ME) before being digitized at a sampling rate of 40 kHz (model AD2; TDT). The digitized signal was uploaded to the computer via a second digital signal-processing card (AP2 Multi-Processor DSP card; TDT). Custom-made software calculated the time of occurrence of the spikes and displayed poststimulus time histograms (PSTHs), raster plots, and statistics on the neural responses in real time.

All recordings were obtained from well-isolated single neurons characterized by stable amplitude and consistent shape. Once single-neuron activity was isolated, tone bursts varying in frequency and amplitude were used to determine the best frequency (BF), the frequency at which the lowest sound pressure level (SPL) induced stimulus-locked action potentials, and the minimum threshold at BF. BF s were measured to tenths of kilohertz but are rounded to kilohertz in RESULTS. Since the response of many NLL neurons to BF tones was suppressed by a second, lower-frequency tone, a two-tone stimulus paradigm was used to reveal and then evaluate the underlying interactions. For this, a high-frequency tone at the neuron’s BF was presented 10 dB above its threshold. Then, a second tone was set at a sufficiently high level to reveal suppressive interactions (>65 dB SPL) and systematically varied across frequencies <40 kHz, in 0.5- or 1.0-kHz steps. The test was performed when the two signals were presented simultaneously or, if necessary, when the BF signal was delayed. In neurons for which the LF signal suppressed the response to the BF signal, quantitative measures of delay-sensitive suppression were obtained and compared with the single-tone responses. Rate-level functions were obtained by increasing the sound level of BF tone bursts in 5-dB increments. Initially, we tested suppressive interactions in the range 23–40 kHz, then investigated suppression <23 kHz as this suppression became more apparent. Our later observations suggest that suppression <23 kHz occurs in most if not all NLL neurons.

The evoked activity of single neurons was obtained using a micropipette electrode mounted on a five-barrel pipette (Hayev and Caspary 1980) for iontophoretic application of drugs. The tip of the multibarrel pipette was broken to a diameter of 15–30 μm; the unbroken tip of the single-barrel electrode extended 10–25 μm beyond the multibarrel pipette and was filled with physiological saline (resistance 50–30 MΩ). The center barrel of the multibarrel pipette was filled with 0.9 M NaCl and connected to a sum channel to balance all currents used to apply or retain drugs. The remaining four barrels were filled with the γ-aminobutyric acid type A receptor (GABAAR) antagonist bicuculline (10 mM, pH 3.0; Sigma, St. Louis, MO) and the glycine receptor (GlyR) antagonist strychnine (10 mM, pH 3.0; Fluka, Milwaukee, WI). Bicuculline and strychnine were retained with negative current (∼15 nA) and ejected using positive currents (∼15 to +40 nA). Iontophoresis currents for drug application and retention were established previously, with control tests for current and pH (Nataraj and Wenstrup 2005, 2006).

Each barrel of the multibarrel pipette was connected via silver wire to a microiontophoresis current generator (programmable current generator, model 6400; Dagan, Minneapolis, MN). The current generator controlled the retention and ejection currents for each barrel separately. The piggyback multibarrel electrode assembly was advanced into the brain by a hydraulic micropositioner (model 650; David Kopf Instruments). Drug injection times and ejection currents...
varied depending on the effect of the drug(s) monitored both audiovisually and quantitatively. Low ejection currents were used initially. If no effect was observed, the ejection current was gradually increased. For every current setting, rate-level functions were obtained until no further change in response was observed. With currents used in this study, effects of bicuculline and strychnine could be observed as early as 2 min after application began. Complete or partial recovery could be observed as early as 4 min after drug application was stopped and was dependent on the particular drug, ejection currents, and the duration of drug application.

**Histological reconstruction**

In four animals, 14 recording sites were marked by iontophoretic deposit of a neural tracer; biotinylated dextran amine (BDA; MW 10,000, 10% in saline; Molecular Probes, Eugene, OR); Fluoro-Gold (FG; 4% in saline; FluoroChrome, Englewood, CO); Fluoro-Ruby (FR; tetramethylrhodamine dextran, MW 10,000, 10% in saline; Molecular Probes); or fluorescein dextran (FD; MW 10,000, 10% in saline; Molecular Probes). Tracers were deposited with either a positive or negative current (5–7 µA; variable on–off) for 5–10 min. No more than four sites were marked in each animal.

Animals with tracer injections survived 5–14 days before they were killed. All other animals were killed and perfused after the last recording session. Each animal was killed with an overdose of Fatal Plus (100 mg/kg, ip; Vortech, Dearborn, MI). Following loss of corneal and withdrawal reflexes, the animal was perfused through the left ventricle with 0.1 M phosphate-buffered saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was then removed and stored overnight at 4°C in 4% paraformaldehyde with 30% sucrose. Brains were frozen, sectioned at 50 µm in the transverse plane, and collected into three series. One or two series (to be used for fluorescence) were mounted on gelatin-coated slides and cover-slipped with DPX. One series was mounted on gelatin-coated slides and stained with cresyl violet for cytoarchitecture. In cases with BDA injections, one series was treated with avidin-biotin-peroxidase and then stained with diaminobenzidine enhanced with nickel ammonium sulfate (Adams 1981). The sections were then mounted on gelatin-coated slides, air-dried, and coverslipped with DPX.

The lateral lemniscus and tracer deposit sites were photographed with a Magnifire camera (Optronics, Goleta, CA) mounted onto a Zeiss Axioplan microscope. Adobe Photoshop was used to overlay injection sites onto cresyl violet–stained sections and to outline lateral lemniscal divisions. These were identified by comparison with those described previously in mustached bats (Wenstrup et al. 1999; Zook and Casseday 1982). Electrode tracks from the large multibarrel pipettes were visible in all seven experimental cases. These tracks were microscopically analyzed in cresyl violet–stained sections to verify location within lateral lemniscus nuclei.

**Data analysis**

Quantitative data were obtained for all neurons using 32 repetitions of each stimulus. Many neurons in NLL were spontaneously active (mean ± SD: 32 ± 36 spikes/s). We therefore defined an excitatory response as a 20% increase in spike discharge, within a 10- to 20-ms time window. Suppression of background activity was based on a 20% reduction. Neurons were considered to show LF suppression if the responses to the combined signals, separated by a given delay, were ≥20% lower than the response of the BF signal. The strength of interactions was quantified by an interaction index, where the index = \((R_2 - R_0 - R_1)/(R_2 + R_0 + R_1)\). \(R_0\) represents the neuron’s response to the combination of low- and high-frequency signals, \(R_1\) represents the neuron’s response to the high-frequency signal presented alone, and \(R_2\) represents the neuron’s response to the low-frequency signal presented alone. An interaction index value of −1 corresponds to the strongest suppression. The 20% criterion corresponds to an interaction index value of −0.11. In some neurons, high background discharge affected calculation of the interaction index; this was minimized by analyzing responses within a 10- or 20-ms window that included any responses to the low and high frequencies. We use the term "suppression" to include two types of LF effects on BF responses: 1) inhibition that results from demonstrable neural interactions and 2) suppression that is of uncertain, possibly cochlear, origin.

Suppressive interactions were characterized by the delay at which the interaction was maximum (best delay), the range of delays at which the interaction occurred (delay width), and the maximum strength of the interaction (interaction index). Paired two-tailed t-tests, testing for differences between the control and drug conditions, were performed with an error level of 0.05, corrected for repeated tests. For many measures, average values are reported with the SE.

**RESULTS**

This report is based on responses of 120 well-isolated neurons in the NLL of awake mustached bats. A later section describes criteria for localizing these neurons to individual lateral lemniscal nuclei. Of these 120 neurons, 103 displayed LF suppression of responses to BF tones bursts. We first describe some functional properties of neurons showing LF suppression, then examine whether these two-tone suppressive responses depended on inhibitory mechanisms acting within the NLL.

**Functional properties of neurons displaying LF suppression**

NLL neurons displaying low-frequency suppression had high-frequency BFs in the range 33–104 kHz. Most neurons (74%) displayed a purely excitatory response to BF signals, but 26% showed an excitatory response that was followed by suppression of background activity. Response latencies at 10 dB above threshold were in the range 3–13 ms.

This study focused on LF suppression tuned at least an octave below the BF. Of the 103 neurons showing LF suppression, 96 fit this criterion. As in IC (Nataraj and Wenstrup 2006), we observed three distinct types of LF suppressive responses based on the presence or absence of LF excitation (Fig. 1A). For the majority of suppressive responses, LF signals were purely suppressive (type I, 59%), reducing both background discharge and the response to BF tones. Other suppressive responses were accompanied by LF excitation prior to the suppression (type EI, 28%) or after the suppression (type IE, 13%). To analyze features of the suppression, we plotted spike count as a function of delay, using windowed responses to minimize the influence of background discharge (Fig. 1B). For type I and type IE responses, LF suppression was typically strongest when the BF and LF signal were presented simultaneously (best delay of 0 ms) (Fig. 1C), similar to previous observations in IC neurons (Nataraj and Wenstrup 2006). We did not evaluate best delay in type EI responses because the LF excitation appears to mask strong suppression of the BF response (Nataraj and Wenstrup 2006).

Lower-frequency suppression was usually tuned <30 kHz, but there was a pronounced frequency dependence of the different types (Fig. 1D). For most type I (83%) and type IE (77%) responses, the LF suppression was tuned ≥23 kHz. In contrast, most type EI responses had suppression tuned <23 kHz (83%). In ten neurons for which we obtained quantitative data on suppression over both frequency ranges, we observed both type EI responses tuned <23 kHz and type I or type IE responses tuned >23 kHz. A similar relationship between the
frequency tuning of suppression and these response types has been reported in the mustached bat’s IC (Gans et al. 2009; Nataraj and Wenstrup 2006).

Thresholds of suppressive responses were in the range 27–82 dB SPL, when the BF was set 10 dB above threshold. Below 23 kHz, thresholds for suppression averaged 67.6 ± 0.9 dB SPL (range, 56–82 dB SPL, n = 49). Suppression thresholds in the 23- to 30-kHz range were lower, averaging 51.8 ± 1.9 dB SPL (range 27–78 dB SPL, n = 102). These differences were highly significant (unpaired t-test, P < 0.001). For suppression tuned in each of these frequency bands, the threshold was significantly higher than the threshold of excitation at BF (multiple paired t-test, P < 0.001). For suppression in the lower band, the difference averaged 40.1 ± 3.0 dB SPL. For suppression in the 23- to 30-kHz band, the threshold was 23.0 ± 3.1 dB higher than the BF threshold. These features of suppression correspond to observations in IC neurons and emphasize the distinction between suppression tuned above and below 23 kHz (Gans et al. 2009; Nataraj and Wenstrup 2006).

In the last half of the study, we examined more closely the presence of suppression in both the 10- to 22- and 23- to 30-kHz ranges. Among 54 neurons, 88% displayed suppressive effects with sensitivity peaks in each frequency band. These observations suggest that suppressive responses in both frequency bands are very common in the areas of NLL from which we recorded. (See last section of RESULTS for recording sites.)

Blockade of inhibition in NLL

Using microiontophoretic application of strychnine and bicuculline in NLL, we examined the contributions of GlyRs and
GABA\_Rs to LF suppression of BF responses. Responses of these neurons were examined before, during, and after successful blockade of GABA\_Rs via bicuculline (n = 17 neurons), GlyRs by strychnine (n = 17 neurons), or both (n = 10 neurons). There were two major results. First, blockade of GlyRs, but not of GABA\_Rs, consistently eliminated or greatly reduced LF suppression of BF responses in NLL neurons. Second, GlyR blockade affected only LF suppression that was tuned in the range 23–30 kHz and was of type I or type IE.

**EXAMPLES OF SINGLE NEURONS.** These major results are illustrated by neuronal responses in Figs. 2–4. Figure 2 displays responses of a neuron from the intermediate nucleus of the lateral lemniscus (INLL). In the control condition, PSTHs (Fig. 2A, Control) show a high background discharge (78 spikes/s) that was typical of neurons in INLL. BF tones (52 kHz) elicited a response with a strong onset component, followed by a brief period of suppression of background activity. LF tones at 27 kHz suppressed the background discharge and evoked a weak rebound burst at stimulus offset. When the two signals were presented simultaneously, at 0-ms delay, there was very strong LF suppression of the BF excitatory response. The delay curve (Fig. 2B) shows that the suppression was a sensitive function of delay of the BF signal and was strongest at simultaneous presentation. The grayscale PSTHs (Fig. 2D) clearly show each of these features: background discharge, BF-evoked excitation and suppression (vertical patterns), and LF suppression and rebound discharge (diagonal patterns). The control test typifies a type IE response to the LF stimulus.

**FIG. 2.** LF suppression activated by 27-kHz tone was eliminated by blockade of glycine receptors (GlyRs, by strychnine) but not by γ-aminobutyric acid type A receptors (GABA\_Rs, by bicuculline). Results from a single neuron. A: PSTHs display temporal discharge pattern in response to BF (57 kHz), LF (27 kHz), and combination of BF and LF at 0-ms delay. Loss of LF suppression occurred during GlyR blockade, with or without GABA\_R blockade. Gray rectangles indicate time window for spike counts (numbers at right) in each PSTH. B and C: delay functions reveal that suppression was strongest when the LF was presented simultaneously with BF. This suppression was eliminated during GlyR blockade. D: grayscale PSTHs show that GlyR blockade removed LF suppression of both background activity and the BF spiking response. Neuron recorded in the intermediate nucleus of the lateral lemniscus (INLL).
This pattern did not change during GABA\textsubscript{A}R blockade. Although there were increases in both the background discharge (78 to 97 spikes/s) and BF-evoked response (62 to 77 spikes per 32 stimuli), the LF-evoked suppression of background activity or of the BF response was largely unaffected (Fig. 2A, GABA\textsubscript{A}R Block). Moreover, the delay sensitivity of LF suppression (Fig. 2, B and D) was unchanged. The addition of GlyR blockade to GABA\textsubscript{A}R blockade further elevated background discharge (to 109 spikes/s) and the BF response (to 97 spikes per 32 stimuli; Fig. 2A, GABA\textsubscript{A}R + GlyR Block). The major effect of addition of GlyR blockade is seen in responses to the LF and combination stimuli: both the LF suppression of background activity and the LF suppression of the BF response were significantly weaker (Fig. 2A). Delay-tuned suppression of the BF response was very weak or not apparent (Fig. 2, B and D). After recovery from combined drug application, strychnine alone was applied to block GlyRs. GlyR blockade reproduced all the effects of combined drug application on LF suppression: on suppression of background discharge and BF responses (Fig. 2A, GlyR Block), as well as on the delay-tuned suppression of the BF response (Fig. 2, C and D). Thus in this neuron, GlyRs appeared to mediate all of the major features of LF suppression of BF responses, whereas GABA\textsubscript{A}Rs appeared to play no significant role.

When LF suppression was tuned <23 kHz, neither GlyR nor GABA\textsubscript{A}R blockade affected the suppression. This is illustrated by the VNLL neuron in Fig. 3. This neuron, tuned to a BF of 56 kHz, displayed excitation followed by suppression in response to the BF signal. It responded similarly to an 18-kHz signal at higher sound level (type EI LF response). When the LF signal was presented 10 ms before the BF signal, there was a strong suppression of the BF response (Fig. 3, A–C). The delay function reveals the timing of suppression (Fig. 3, B and C). It is noteworthy that the BF response did not suppress the excitatory response to a delayed LF signal, even though it reduced background discharge for a period after its excitatory response. Thus the LF-evoked suppression and the BF-evoked suppression were not of the same type. Blockade of GABA\textsubscript{A}Rs alone or in combination with GlyRs did not alter the LF suppression of background activity or BF responses (Fig. 3A) and delay sensitivity functions were similarly unaffected (Fig. 3, B and C).

**FIG. 3.** Suppression by 18-kHz tone was not eliminated by application of GABA\textsubscript{A}R or GlyR blockers in this single neuron. A: PSTHs display temporal discharge pattern in response to BF (56 kHz), LF (18 kHz), and combination of BF and LF tones at 10-ms delay. B: delay functions reveal that suppression was strongest when the LF was presented 10 ms before the BF. C: grayscale PSTHs show that GABA\textsubscript{A}R and GlyR blockers increased background spiking activity but did not influence the LF-suppressive response. Neuron recorded in the ventral nucleus of the lateral lemniscus (VNLL).
The neuron in Fig. 3 displayed LF suppression tuned to two distinct frequencies: responses in Fig. 3 show suppression that was tuned to 18 kHz, type EI, and insensitive to blockade of inhibition. This same neuron also showed LF suppression tuned to 28 kHz that was different in both the type of suppression and the sensitivity to inhibitory blockers. This is illustrated in Fig. 4. In control recordings, the 28-kHz signal suppressed background discharge (type I). Furthermore, when the 28-kHz signal was presented simultaneously with the BF signal, strong suppression of the BF response occurred (Fig. 4A). The suppression was strongest when the two signals were presented simultaneously (Fig. 4, B and C). These responses to the 28-kHz signal were thus different from those evoked by the 18-kHz signal illustrated in Fig. 3.

GABAAR blockade had similar results for 18- and 28-kHz suppression. Thus it increased background activity and spike discharge in response to the BF signal, while having no effect on 28-kHz suppression of either background activity or of the BF response (Fig. 4A). Both the delay curve and the delay PSTH were similarly unaffected (Fig. 4, B and C).

Effects of GlyR blockade were very different for 28-kHz suppression compared with 18-kHz suppression. With the addition of GlyR blockade, 28-kHz suppression of the background activity was reduced but still present (Fig. 4A, GABAAR + GlyR Block). However, the ability of the 28-kHz signal to suppress the BF response was eliminated across all presented delays (Fig. 4, B and C). These results support the main observations that GlyRs but not GABAARs contributed to LF suppression, but only when that suppression was tuned in the 23- to 30-kHz range. The results illustrated in Figs. 3 and 4 show that the differential effects of drug application and frequency can be observed for the responses of a single neuron.

**POPULATION DATA.** Successful blockade of GABAARs and/or GlyRs was obtained in 24 neurons. There was a marked difference in the effects of GlyR blockade on suppression tuned above and below 23 kHz (Fig. 5A). Consistently strong reduction of LF suppression occurred only during GlyR blockade of suppression tuned in the range 23–30 kHz (Fig. 5B). Suppression was eliminated in 12 of 15 neurons and significantly reduced in the other 3 neurons. This was true when GlyRs were blocked separately (solid lines, Fig. 5B) or in combination with GABAAR blockade (dashed lines). Separate GABAAR blockade of suppression tuned to 23–30 kHz had no consistent effect on the suppression and never eliminated it (Fig. 5C). For suppression tuned <23 kHz, neither GlyR blockade nor GABAAR blockade had any consistent effect (Fig. 5, D and E).

**FIG. 4.** Suppression by 28-kHz tone was eliminated by addition of GlyR blockade. (Note: Fig. 3 shows suppression tuned to 18 kHz for this same neuron.) A: PSTHs display temporal discharge pattern in response to BF (56 kHz), LF (28 kHz), and combination of BF and BF at 0-ms delay. LF suppression persisted during GABAAR blockade, but was eliminated with addition of GlyR blocker (GABAAR + GlyR Block). B: delay functions reveal that delay-tuned suppression was strongest when the LF was presented simultaneously with the BF. This suppression was eliminated during GABAAR + GlyR blockade. C: grayscale PSTHs show that addition of GlyR blocker removed LF suppression of both background activity as well as the BF spiking response. Note that drug application did not have an effect on the 18-kHz suppression displayed by this neuron (Fig. 3).
RECORDING SITES. For 14 neurons, recording sites were marked by tracer deposits and histologically localized to the NLL (e.g., Fig. 6). In several additional penetrations, the multibarrel penetration track aided identification. For the remainder, we assessed location based on electrode depth, background discharge, latency, and depth-related changes in frequency tuning. Response latencies were in the range 3–13 ms (mean, 5.9 ms), consistent with previous recordings in NLL (Portfors and Wenstrup 2001). In most penetrations, the electrode passed through the caudal IC with tuning to 55–60 kHz laterally or 60–120 kHz more medially. The electrode then passed through an area just ventral to IC where no responses could be recorded. At depths >2,000–2,400 μm, the electrode passed into the NLL. Major shifts in BF characterized transitions from the dorsal nucleus (DNLL) to INLL, INLL to the ventral nucleus of the lateral lemniscus (VNLL), and between the multipolar and columnar divisions of VNLL (VNLLm and VNLLc, respectively). These are consistent with previous physiological recordings (Portfors and Wenstrup 2001) and axonal transport studies (Wenstrup et al. 1994, 1999; Zook and Casseday 1985). Because most penetrations were placed more laterally to include both INLL and VNLL, they typically

FIG. 5. Glycinergic inhibition played a major role in the creation of LF suppression tuned to 23–30 kHz. A: change in strength of suppression (interaction index) after GlyR blockade as a function of frequency tuning of suppression. Effect of GlyR blockade is much stronger for suppression tuned >23 kHz. B–E: plots display strength of suppression (interaction index) during control, receptor blockade, and recovery experimental conditions. B and C: LF suppression tuned to 23–30 kHz, D and E: LF suppression tuned <23 kHz. Maximal suppression = −1 interaction index, minimal suppression = −0.11. Values more positive than +0.09 indicate facilitation. Gray boxes indicate values with no suppression or facilitation, as defined in METHODS. Dashed lines (B, D) show responses of neurons for which GlyR blockers were added to GABA<sub>AR</sub> blockers. For these neurons, “Control” values are those obtained during GABA<sub>AR</sub> Block, prior to addition of GlyR blockers.

LF Suppression: 23-30 kHz

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<th>Recovery</th>
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LF Suppression: <23 kHz

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Suppression eliminated
Suppression not eliminated
missed DNLL. No quantitative data were obtained from neurons localized to DNLL. Based on these analyses, we localized recording sites of 80 neurons to INLL, including 15 neurons studied pharmacologically. Twenty-three neurons were localized to VNLL, including 9 neurons studied pharmacologically. Of these VNLL neurons, 15 displayed a tonic discharge pattern observed only in the VNLLm in the mustached bat (Portfors and Wenstrup 2001). The 2 neurons with onset discharge patterns were also localized to VNLLm due to their proximity to the INLL/VNLL border.

**DISCUSSION**

This report examined the contribution of lateral lemniscal nuclei (NLL) to spectral integration in the ascending auditory pathway. Here we focused on two types of spectral integration that feature suppressive interactions tuned far from a neuron’s best (or characteristic) frequency. For one type, suppression is tuned to the frequency band 23–30 kHz and may have specific functions associated with analyzing sonar echoes or communication signals. Blockade of GlyRs by strychnine eliminates this suppression, indicating that it is the result of glycinergic inhibition acting within NLL. These interactions likely contribute to combination-sensitive inhibition observed in recordings of IC, medial geniculate body, and auditory cortex. A second type of LF suppression is generally tuned to frequencies <23 kHz and it is often associated with an initial excitatory response to those frequencies. This suppression is not eliminated by blockade of either GABA_A Rs or GlyRs in NLL, suggesting that it originates below the NLL. We believe this suppression is of cochlear origin. Regardless of origin, however, both types of spectral interactions shape the response of auditory neurons to complex sounds.

*Spectral integration performed by NLL neurons*

This study finds that most NLL neurons display at least one form of LF suppression. This is significantly more common than previously reported in the mustached bat (Portfors and Wenstrup 2001) or other species. It is likely that we observed more suppressive interactions for two reasons. First, the previous study in mustached bats focused primarily on suppressive interactions tuned in the 23- to 30-kHz band. The present study extends these findings by describing suppressive responses <23 kHz, which are observable only at significantly higher levels than needed to observe 23- to 30-kHz suppression. When closely examined, suppression <23 kHz appears to occur in most or all NLL neurons. Second, the present study examined more closely the regions of NLL, especially INLL, where combination-sensitive inhibitory interactions may be concentrated (Portfors and Wenstrup 2001).

The present results indicate that one form of LF suppression, tuned to frequencies <23 kHz, is not created within NLL but is likely inherited from inputs to NLL. The inability of GlyR and GABA_A blockers to eliminate or substantially alter this suppression, even when the blockers eliminate another form of suppression, argues strongly for an origin below the NLL. This is supported by observations of such suppressive responses in cochlear nuclei (Marsh et al. 2006). Although we cannot rule out an origin in cochlear nuclei, this suppression and its associated excitation, as described in IC neurons (Gans et al. 2009; Nataraj and Wenstrup 2006), bear the hallmarks of two-tone suppression and associated excitatory responses observed in high BF AN fibers (Arthur et al. 1971; Delgutte 1990; Joris et al. 1994; Kiang and Moxon 1974; Sachs and Kiang 1968), features that originate in the cochlea (Ruggiero et al. 1992; Temchin et al. 1997). These features include broad LF sensitivity, high thresholds exceeding 60–65 dB SPL, associated excitatory responses at some sound levels, and suppression that lasts the duration of the LF stimulus.

This study expanded on previous work (Portfors and Wenstrup 2001) to provide more detailed information about a second type of LF suppression that occurs in INLL and VNLLm. For many NLL neurons (having BFs ≥50 kHz and

**FIG. 6.** Recording sites were located within NLL. Photomicrographs of transverse sections show examples of tracer deposits that mark the location of recording sites showing LF suppression. A: FluoroRuby injection site (black). B: Nissl-stained section of a biotinylated dextran amine (BDA) injection site (black). Lines indicate the path of the electrode. Images were photographed in color and converted to grayscale. Dorsal is up, lateral is left (in A) or right (in B). CG, central gray; DC, dorsal cortex of the inferior colliculus; DNLL, dorsal nucleus of the lateral lemniscus; IC, central nucleus of the inferior colliculus; INLL, intermediate nucleus of the lateral lemniscus; VNLL, ventral nucleus of the lateral lemniscus.
mostly within frequency ranges associated with higher harmonics of the echolocation signal), spike discharge to BF tones is suppressed by low frequencies in the 23- to 30-kHz range, a range associated with the first harmonic of the bat’s echolocation signal. The timing of this suppression appears closely matched to BF-evoked excitation, so that the maximum suppressive effect is usually observed when the BF and LF signals are presented simultaneously. Overall, these response properties correspond closely to those in IC neurons that we have termed “combination-sensitive inhibition” (Leroy and Wenstrup 2000; Mittmann and Wenstrup 1995; Portfors and Wenstrup 1999).

The major finding of this study is that this second form of LF suppression—combination-sensitive inhibition—depends on LF inhibitory (glycinergic) input acting within VNLLm and INLL. Blockade of GABA_ARs has no consistent effect on this suppression, but GlyR blockade always eliminates or greatly reduces it. Further, the effect of GlyR blockade is selective for suppression in the 23- to 30-kHz range. Among neurons that show both this and the other form of suppression, only the 23- to 30-kHz suppression is eliminated with GlyR blockade (e.g., compare Figs. 3 and 4). These results strongly suggest that this form of suppression is created within the NLL and are supported by previous work showing that combination-sensitive inhibition is rare in cochlear nuclei (Marsh et al. 2006). Based on both the past and present work, we conclude that INLL and some VNLLm neurons perform a specific form of frequency integration in which the presence of acoustic energy in the 23- to 30-kHz band excites an LF-tuned glycinergic input to these neurons. If timed correctly, this input will reduce the responsiveness of these NLL neurons to sounds in their excitatory receptive fields. The source of this glycinergic input is most likely to originate in the medial or lateral nuclei of the trapezoid body (Glendenning et al. 1981; Huffman and Covey 1995; Sommer et al. 1993; Spangler et al. 1985; Zook and Casseday 1987). However, local glycinergic circuits within INLL/VNLL may provide another possible source of glycinergic input (Nayagam et al. 2005).

At present it is unclear whether similar spectral interactions occur in NLL of other species, although INLL and VNLL are known to receive inputs from glycinergic nuclei, including the medial and lateral nuclei of the trapezoid body (Glendenning et al. 1981; Huffman and Covey 1995; Irfan et al. 2005; Kelly et al. 2009; Sommer et al. 1993; Spangler et al. 1985; Yavuzoglu and Wenstrup 2009; Zook and Casseday 1987). However, few studies have attempted to assess the role of inhibitory spectral interactions in NLL through presentation of more than single tonal stimuli or through blockade of inhibitory neurotransmitters. The most closely related studies in different species have yielded varied results. In rufous horseshoe bats (Rhinolophus rouxi), Metzner and Radke-Schuller (1987) reported evidence of spectrally distant inhibition that suppresses background activity in NLL neurons. In the free-tailed bat (Tadarida brasiliensis), Xie and colleagues (2005) addressed issues similar to the present study but reported very different results. In both INLL and DNLL, they found that blockade of inhibition had virtually no effect on the width of tonal excitatory tuning curves or on the responses to a suite of the bat’s vocal signals. They concluded that inhibition in INLL and DNLL does not contribute to frequency tuning or responses to complex sounds. Surprisingly, even the discharge rate of INLL neurons is generally unaffected by blockade of GABAergic or glycinergic inhibition. In contrast, we often observe increases in spike discharge with GABA_A or GlyR blockade (e.g., Figs. 2–4) and 23- to 30-kHz inhibition is always susceptible to GlyR blockade. Given the experience of both laboratories in microiontophoretic methods, species differences may be the best explanation for the dramatically different results among INLL neurons. We thus concur with Xie and colleagues (2005) that substantial species differences exist in auditory processing within INLL. Similarly, we believe that differences in spectral tuning (width or presence of multiple sensitivity peaks) or temporal responses properties (e.g., temporal pattern of discharge) among VNLL neurons represent adaptations in auditory processing among chiropteran (Covey and Casseday 1991; Metzner and Radke-Schuller 1987; Portfors and Wenstrup 2001; Xie et al. 2005) and other mammalian groups (Batra and Fitzpatrick 1999; Zhang and Kelly 2006).

Distant spectral inhibition underlies various forms of complex sound processing

The present results support our hypothesis that spectrotemporal processing within INLL and possibly VNLL provides the basis for specific response properties observed in IC neurons. We propose that IC neurons displaying two kinds of spectrally integrative responses inherit the inhibitory combination-sensitive response property from neurons in INLL and possibly VNLLm (Fig. 7). One type of IC response, comprising about 25% of neurons in high-frequency regions of the IC (Nataraj and Wenstrup 2006), displays two-tone frequency interactions that are nearly identical to the 23- to 30-kHz inhibition observed within NLL of this study (Fig. 7B) (Mittmann and Wenstrup 1995; Nataraj and Wenstrup 2006; O’Neill 1985; Portfors and Wenstrup 1999). A second type (Fig. 7C), including about 20% of the same population (Nataraj and Wenstrup 2005), shows both inhibition and facilitation by 23- to 30-kHz sounds, but at different timing relative to the BF signal (Mittmann and Wenstrup 1995; Nataraj and Wenstrup 2005; Portfors and Wenstrup 1999). For this second type, the inhibition occurs when the two signals are presented simultaneously, whereas the facilitation occurs when the BF signal is delayed by several milliseconds.

Our proposal is that the 23- to 30-kHz inhibition displayed by both types of IC neurons is the result of a direct excitatory projection from the inhibitory combination-sensitive neurons in INLL and perhaps VNLLm (Fig. 7D). There are several lines of evidence that support the inheritance hypothesis. First, many features of 23- to 30-kHz inhibition are similar in NLL and IC neurons, including the best inhibitory delay of 0 ms, thresholds of LF inhibition, and relationship to LF excitatory responses (Gans et al. 2009; Nataraj and Wenstrup 2006; Portfors and Wenstrup 1999). Second, among combination-sensitive IC neurons of both types, the 23- to 30-kHz inhibition is not eliminated by local application of GlyR or GABA_A blockers (Nataraj and Wenstrup 2005, 2006). Third, over half of IC neurons showing 23- to 30-kHz inhibition do not display LF-evoked inhibitory postsynaptic potentials (Peterson et al. 2008). Both of these points suggest that the inhibition originates below the IC. Fourth, INLL and VNLLm project strongly to tracor deposit sites in IC that display inhibitory combination-sensitive responses as shown in Fig. 7B (Wenstrup et al. 1999).
Fifth, many and perhaps most INLL neurons appear to be excitatory. This last point requires close inspection, since it is often presumed that the VNLL/INLL complex provides primarily inhibitory projections to IC. In the mustached bat, the majority of neurons in INLL are unlabeled by glycine or GABA immunocytochemistry, unlike VNLL and DNLL neurons observed in the same histological sections (Winer et al. 1995). Regions corresponding to INLL in rat and cat, sometimes considered to be the most dorsal part of VNLL, also show significant numbers of presumptive excitatory neurons (Riquelme et al. 2001; Saint Marie et al. 1997). It is less clear whether neurons in VNLLm are excitatory (Winer et al. 1995). Although not definitive, these lines of evidence provide strong support for the hypothesis that IC neurons displaying inhibitory combination-sensitive responses inherit these responses from excitatory inputs from INLL (Fig. 7D).

For facilitated combination-sensitive responses in IC, our proposal is that the INLL excitatory input contributes to the excitatory drive in response to BF signals and creates the LF inhibition of the BF response (Fig. 7D, bottom right). However, our previous work shows that the facilitatory interaction depends on a separate mechanism. Specifically, glycineric inputs tuned both to the 23- to 30-kHz input and to the neuron’s BF create the facilitatory interaction in IC neurons, possibly through a mechanism of postinhibitory rebound (Fig. 7D, bottom right, glycineric inputs) (Nataraj and Wenstrup 2005; Sanchez et al. 2008; Wenstrup and Leroy 2001). For these higher-order IC neurons, it is a paradox that the inhibitory combination-sensitive response property arrives via excitatory brain stem inputs, whereas the facilitatory interaction depends on “inhibitory” brain stem inputs. This paradox argues for caution when interpreting both the location of origin of higher-order response properties and the neurotransmitters that may contribute.

Functionally, these combination-sensitive IC neurons show spectral and temporal responses appropriate for analyzing the delay between emitted biosonar signals and returning echoes. We and others have speculated that the combination-sensitive inhibition is separate from the lateral inhibitory suppression of a neuron’s response to excitatory sounds that may contribute. Functionally, these combination-sensitive IC neurons show spectral and temporal responses appropriate for analyzing the delay between emitted biosonar signals and returning echoes. We and others have speculated that the inhibition by 23- to 30-kHz signals of a simultaneous signal near the neuron’s BF eliminates its responsiveness to emitted sonar signals (Mittmann and Wenstrup 1995; Olsen and Suga 1991; Portfors and Wenstrup 1999). The proposed contribution of INLL-generated response properties to these combination-sensitive IC responses is the only specific proposal of which we are aware that relates processing in INLL to specific IC responses.

More broadly, the spectrotemporal interactions documented here in NLL neurons may underlie tuned, spectrally distinct suppression of a neuron’s response to excitatory sounds that occurs in many neurons in the auditory midbrain and forebrain. Sutter and colleagues (1999) showed that many neurons in cat primary auditory cortex (AI), particularly in dorsal AI, display tuned inhibition that is separate from the lateral inhibitory bands. These multiple inhibitory frequency bands are thought to contribute to analyses of spectral shape of complex stimuli. The temporal properties of these inhibitory bands may dictate responsiveness to sequences of complex sounds (Brosch and Schreiner 1997, 2000). The temporal features of distant spectral interactions may vary substantially across neurons (Brosch and Schreiner 1997) or across the individual spectral response elements that constitute a neuron’s complex receptive field (e.g., Gans et al. 2009; present study).

Tuned suppressive spectral interactions are associated with analyses of sound location and vocal signals. Imig and colleagues (Imig et al. 1997; Poirier et al. 2003; Samson et al.
In mustached bats, suppressive interactions in both 10- to 22- and 23- to 30-kHz bands will alter neural responsiveness to several social vocalizations that have energy within these bands. Such suppressive interactions, in mustached bats or other species, appear to limit neural responsiveness to social vocalizations with energy in the suppressive-frequency band (Leroy and Wenstrup 2000; Rauschecker et al. 1995)—predictions of suppressive interactions would be more accurate when these inhibitory bands are taken into account (Holstrom et al. 2007). The present results establish that these types of analyses may play a substantial role.

**Acknowledgments**

We thank the Auditory Neuroscience Group at Northeastern Ohio Universities College of Medicine for discussion of the data, C. Grose for assistance with the manuscript, and the Wildlife Section of the Ministry of Agriculture, Land and Marine Resources of Trinidad and Tobago for permission to export bats.

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**Grants**

This work was supported by National Institute on Deafness and Other Communication Disorders (NIDCD) Grants R01 DC-00937 to J. J. Wenstrup and NIDCD/U.S. Public Health Service National Research Service Award F32 DC-007786 to D. C. Peterson.

**References**


