Responses of Neurons in the Rat’s Ventral Nucleus of the Lateral Lemniscus to Monaural and Binaural Tone Bursts

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Zhang, Huiming and Jack B. Kelly. Responses of neurons in the rat’s ventral nucleus of the lateral lemniscus to monaural and binaural tone bursts. J Neurophysiol 95: 2501–2512, 2006. First published January 4, 2006; doi:10.1152/jn.01215.2005. Responses to monaural and binaural tone bursts were recorded from neurons in the rat’s ventral nucleus of the lateral lemniscus (VNLL). Most of the neurons (55%) had V- or U-shaped frequency-tuning curves with a single clearly defined characteristic frequency (CF). However, many neurons had more complex, multipeaked tuning curves (37%), or other patterns (8%). Temporal firing patterns included both onset and sustained responses to contralateral tone bursts. Onset and sustained responses were distributed along the dorsoventral length of VNLL with no indication of segregation into different regions. Onset neurons had shorter average first-spike latencies than neurons with sustained responses (means, 8.3 vs. 14.8 ms). They also had less jitter, as reflected in the SD of first-spike latencies, than neurons with sustained responses (means, 0.59 and 4.2 ms, respectively). The extent of jitter decreased with an increase in stimulus intensity for neurons with sustained responses, but remained unchanged for onset neurons tested over the same range. Many neurons had binaural responses, primarily of the excitatory/inhibitory (EI) type, widely distributed along the dorsoventral extent of VNLL. Local application of the AMPA receptor antagonist NBQX reduced excitatory responses, indicating that responses were dependent on synaptic activity and not recorded from passing fibers. The results show that many neurons in VNLL have a precision of timing that is well suited for processing auditory temporal information. In the rat, these neurons are intermingled among cells with less precise temporal response features and include cells with binaural as well as monaural responses.

INTRODUCTION

The ventral nucleus of the lateral lemniscus (VNLL), a prominent structure in the auditory brain stem, consists of a discrete collection of neurons located along the pathway from cochlear nucleus to inferior colliculus. The VNLL receives major afferent projections from the contralateral ventral cochlear nucleus (VCN), the ipsilateral medial nucleus of the trapezoid body (MNTB), and other ipsilateral periolivary nuclei, but does not receive projections from the medial or lateral superior olives, two nuclei that have been shown to be important for binaural processing in the auditory system (Adams 1997; Fraia and Ostwald 1988; Glendenning et al. 1981; Huffman and Covey 1995; Oertel and Wickersberg 2002; Schofield and Cant 1997; Smith et al. 1991, 1993a,b; Spangler et al. 1985; Vater et al. 1997; Warr and Beck 1996; Zook and Casseday 1985). For this reason, the VNLL is considered to be a nucleus dedicated primarily to processing information about the temporal or spectral quality of auditory stimuli rather than the binaural disparities that provide cues for the localization of sounds in space (Covey and Casseday 1991; Oertel and Wickersberg 2002).

The anatomical organization of VNLL varies considerably among the mammalian species for which data are available. For example, echolocating bats have a hypertrophied and highly differentiated VNLL that can be subdivided into columnar and multipolar areas based on cell morphology (Covey and Casseday 1986; Huffman and Covey 1995; Vater et al. 1997). Neurons in the columnar region are uniformly small and round with thick dendrites that resemble those of bushy cells in the cochlear nucleus. Neurons in the multipolar region are less densely packed and have multiple dendrites that spread out from the cell body with no consistent orientation. Different species of echolocating bats, including mustached bats, big brown bats, and horseshoe bats, have slightly different patterns of organization of these VNLL subdivisions (Covey and Casseday 1995). In contrast, some nonecholocating mammals, including rats, cats, guinea pigs, and mice, have a relatively undifferentiated VNLL with bushy cells and stellate (i.e., multipolar) cells mixed together within the same architectonic boundaries (Merchán and Berbel 1996; Zhao and Wu 2001).

Electrophysiological studies have been conducted in several species including echolocating bats (Covey and Casseday 1991; Huffman et al. 1998a,b; Metzner and Radtke-Schuller 1987), cats (Adams 1997; Aitkin et al. 1970; Guinan et al. 1972a,b), and rabbits (Batra and Fitzpatrick 1997, 1999, 2002). In the bat, neurons in the columnar area of VNLL show exceptionally high temporal precision in their response to the onset of a sound, suggesting a specialized role in representing the timing of acoustic events. In contrast, neurons in the multipolar area exhibit both onset and sustained responses to sounds with various temporal firing patterns and latencies (Covey and Casseday 1991). The responses in the undifferentiated VNLL of rabbits appear to have less temporal precision than those reported for bats (Batra and Fitzpatrick 1999).

Electrophysiological studies have yielded mixed results regarding the frequency organization of VNLL in different species. In the big brown bat, neurons in the columnar area form a dorsal to ventral topographic representation of sound frequencies, whereas those in the multipolar region display a rough tonotopic organization in which high frequencies are represented centrally and lower frequencies in concentric surrounding layers (Covey and Casseday 1986, 1991). The horseshoe bat also has a separate and distinct tonotopic organization...
within each subdivision of VNLL (Metzner and Radtke-Schulzer 1987). In cats, Aitkin et al. (1970) found a low- to high-frequency progression along the dorsal to ventral axis of the VNLL. On the other hand, neither Guinan et al. (1972a,b) nor Adams (1997) found any evidence of a tonotopic organization in the cat’s VNLL. Also, Glendenning and Hutson (1998) found no tonotopic gradient in the cat’s VNLL using 2-DG mapping methods.

There have been no electrophysiological studies of the frequency organization of the rat’s VNLL. However, neither metabolic mapping with 2-DG nor sound-induced c-fos expression has suggested the presence of a tonotopic gradient in the rat’s VNLL (Friauf 1992; Saint Marie et al. 1999). Also, retrograde tract tracing from frequency-specific regions of the inferior colliculus results in distributed labeling along the length of VNLL, suggesting a diffuse frequency representation (Kelly et al. 1998; Malmierca and Merchán 2004; Merchán and Berbel 1996).

Whether the neurons in VNLL are sensitive to binaural stimuli is also a controversial issue. Covey and Casseday (1991) found that neurons in the big brown bat’s VNLL were driven exclusively by contralateral stimulation and were not affected by binaural stimulation. In this species, binaural responses were found in a separate nucleus located medial to the VNLL within the lateral tegmentum (Covey 1993). In rabbits, Batra and Fitzpatrick (1997, 2002) found that many neurons in VNLL, particularly along its medial edge, responded to binaural stimulation and were sensitive to binaural time disparities. Binaural responses have also been recorded from the cat’s VNLL. Aitkin et al. (1970) reported that a small percentage (11%) of neurons in the cat’s VNLL respond to binaural stimulation. Guinan et al. (1972a,b) found a much larger percentage (>60%) of binaural neurons in the cat’s VNLL, including those with excitatory and/or inhibitory (EE and EI) interactions. Binaural neurons were found interspersed among monaural neurons (Adams 1997; Aitkin et al. 1970; Guinan et al. 1972a,b). There have been no published reports on binaural response properties of neurons in the rat’s VNLL.

Intracellular recordings have been made from the rat’s VNLL to examine the intrinsic and synaptic properties of its neurons (Nayagam et al. 2005; Wu 1999; Zhao and Wu 2001). However, a more systematic examination of responses to acoustic stimulation is needed to determine the function of the rat’s VNLL in auditory processing.

The purpose of the present study was to determine the response characteristics of neurons in rat’s VNLL, with monaural and binaural tone burst stimuli. We examined temporal firing patterns, frequency-tuning curves, and the sensitivity of neurons to binaural level differences. The results provide information about the possible role of the VNLL in auditory processing and reveal both similarities and differences in response properties between rats and other mammalian species.

**METHODS**

**Animal preparation**

Experiments were conducted on 33 male adult Wistar albino rats (*Rattus norvegicus*) (250–500 g) obtained from Charles River Canada, St. Constant, Quebec, Canada. The external ears and tympanic membranes were examined with an otoscope and determined to be free of infection or other abnormalities. Surgical anesthesia was induced initially by combined injection of ketamine hydrochloride [60 mg/kg, administered intramuscularly (im)] and xylazine hydrochloride (10 mg/kg, im). Supplemental injections of ketamine hydrochloride (20 mg/kg, im) and xylazine hydrochloride (3.3 mg/kg, im) were made as needed throughout the course of an experiment to maintain a state of areflexia.

A midline incision was made in the scalp and the skin, muscles were retracted laterally, and a small craniotomy was made on the left side of the skull to permit insertion of an electrode into the VNLL. Small bone screws were placed in the skull and fixed to a stainless steel rod with dental acrylic. The rod was then attached to a stereotaxic instrument (Kopf Instruments, Tujunga, CA) to hold the head firmly in place while leaving the external ear canals free for insertion of earphone drivers. Recordings were made with the rats inside a single-wall sound-attenuated booth (Eckel Industries, Morrisburg, Ontario, Canada).

All procedures were approved by the Carleton University Animal Care Committee and were in accordance with the guidelines of the Canadian Council on Animal Care.

**Sound stimulation**

Sounds were presented to a rat through a pair of sealed headphone assemblies (Beyerdynamic DT 48, Heilbronn, Germany), each of which was connected to a hollow speculum that was inserted into the rat’s external meatus. The acoustic waveforms were generated digitally by MALab 881 software controlled by a Macintosh G3 computer. The sound generation and data acquisition system (MALab 881) was designed and developed by Dr. Malcolm Semple (Center for Neural Science, New York University) and Steve Kaiser (University of California, Irvine) and produced by Kaiser Instruments.

The acoustic stimuli were noise bursts and tone bursts with a 100-ms duration and 10-ms rise/fall times. Noise bursts presented to the ear contralateral to the recording site were used as search stimuli. Tone bursts presented to the contralateral ear were used in the examination of a single neuron’s temporal firing pattern and frequency-tuning characteristics. Tone bursts presented dichotically were used to characterize binaural responses.

The sound-generating system was calibrated over a frequency range of 100–40,000 Hz using a condenser microphone (Brüel & Kjær 4134). The acoustic response of the headphone assembly was adjusted to provide a constant sound pressure over the range from 500 Hz to 40 kHz.

**Recording electrodes**

Two types of electrode were used in the present study. In most cases, we used a single-barrel glass pipette to record action potentials from single neurons in the VNLL. The tip of the pipette was 1.5–2.0 μm in diameter. The electrode was filled with 3 M potassium chloride or 1.5% biocytin in 0.5 M potassium chloride.

In some cases, we used a piggyback multibarrel glass electrode assembly (Havey and Caspary 1980) to record spikes from single neurons while releasing microiontophoretically an antagonist specific to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors at the recording locus. The purpose of releasing 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX) was to test whether the recordings were made from local VNLL neurons or from fibers of passage from lower brain stem structures. Because the AMPA receptor is the predominant type of excitatory neurotransmitter receptor in the VNLL (Irfan et al. 2005), an injection of NBQX would be expected to reduce or eliminate responses to sounds if the responses were generated by local synapses on VNLL neurons. The electrode was fabricated by fixing a single-barrel micropipette (for recording neural responses) to a five-barrel glass pipette (for drug delivery) with cyanoacrylate glue (Viachem, Montreal, Quebec, Canada) at an angle of about 20°.
pipette was the same as that described above, and the tip of the five-barrel pipette was 8–10 μm in diameter. Two barrels of the five-barrel pipette were filled with NBQX (5 mM, pH 9.0, Sigma) in a vehicle of 165 mM sodium chloride. The remaining three barrels were filled with 165 mM sodium chloride to maintain an electrical balance across the injection pipette tip.

The electrode assembly was driven remotely by a hydraulic micropositioner (model 650, Kopf Instruments). The barrels of the multibarrel pipette were connected to iontophoresis pumps of a Neurophore-BH-2 micro-iontophoresis system (Biomedical Systems, St. Louis, MO). Neural activity recorded by the single-barrel pipette was amplified by an EX 4–400 Quad differential amplifier (Dagan, Minneapolis, MN) and monitored audiovisually. Neural responses were digitized and sampled using an A/D converter and MALab 881 software. The occurrence times of spikes were recorded with a resolution of 1 μs, stored on computer, and processed later with standard database and graphics software.

Recording procedure

During a recording session, the left VNLL was approached obliquely with the electrode in the coronal plane and tilted laterally by 30° relative to the sagittal plane. Stereotaxic coordinates were referenced to a point 6.2–6.7 mm lateral and 0.5–0.9 mm rostral to lambda. The electrode was lowered into the brain to a depth between 6.3 and 8.8 mm while responses to noise bursts presented to the right ear were monitored. In cases in which a multibarrel pipette assembly was used, a retaining current of +10 nA was applied to the pipette barrels containing NBQX as the electrode was lowered into the brain. The retaining current prevented leakage of the drug during the cell-search and predrug recording phases of the experiment.

Single-unit activity was recognized as spikes with constant waveform and amplitude. A window discriminator was used to isolate spikes from background activity. After an auditory neuron had been identified, tone bursts were presented to the right ear at various frequencies and amplitudes to determine the neuron’s frequency-tuning curve and the characteristic frequency (CF, the frequency at which a cell showed the lowest threshold). A threshold was defined as the lowest stimulus level at which a neuron generated acoustically driven spikes on at least three of five trials of tone burst presentation. Tone bursts at the cell’s CF and at various sound pressure levels were then used to determine a rate-level function.

After monaural responses were recorded, we used dichotic tone bursts at the cell’s CF to determine its binaural response characteristics and to establish the sensitivity to interaural-level differences. Tones were delivered to the contralateral ear at 10 to 20 dB above threshold, whereas tones of the same frequency were delivered to the ipsilateral ear at systematically increasing sound pressure levels. Tone bursts were presented 20 times at a rate of one per second to generate summed neural responses for plotting both rate-level functions and interaural-level difference curves.

In 17 neurons, the AMPA receptor antagonist NBQX was delivered microiontophoretically to determine whether neural responses were dependent on local synapses. The currents used to release the drug were ~20 to ~30 nA. The drug effect was monitored by repeatedly recording responses to monaural and/or dichotic tone bursts during microiontophoresis. Typically, the drug began to show an effect in about 1 to 2 min and reached a plateau in about 5 to 8 min after the onset of drug release. Neural responses to monaural and dichotic tone bursts were recorded when the drug effect reached a plateau. The drug release was then terminated and the holding current was reapplied. Recovery was observed by monitoring the level of activity after the drug release was discontinued.

**Histology**

The final recording locus in a rat’s VNLL was marked by iontophoreric injection of biocytin hydrochloride. The current used for injection was a train of 1-μA positive pulses with a pulse duration of 7 s and an interpulse interval of 7 s. Each injection lasted for 15 min.

Twenty to 30 min after an injection, the rat was intraperitoneally administered an overdose of sodium pentobarbital and was transcardially perfused with 4% paraformaldehyde in 0.2% picric acid. The brain was extracted, frozen, and sectioned at 40 μm at the frontal plane. The slices were thoroughly washed with phosphate buffer and reacted with avidin–biotin–peroxidase complex (Vectastain ABC kit, Vector Laboratories, Burlington, CA). Our histological results revealed that the marked recording loci were consistently within the VNLL.

**Data analysis**

For each neuron, we used a poststimulus time histogram (PSTH) to reflect the temporal characteristics of firing elicited by a tone burst presented to the contralateral ear. Each PSTH was based on responses evoked by 20 presentations of a tone burst at a given stimulus intensity. The temporal characteristics were also examined by calculating the average first-spike latency and the SD of the first-spike latencies based on responses to the 20 tone-burst presentations.

We used a frequency-tuning curve to reflect the frequency selectivity of a VNLL neuron. To evaluate the narrowness of frequency tuning, we calculated a Q10, i.e., the ratio between the CF and the width of the frequency-tuning curve 10 dB above the threshold at CF. For a neuron showing two or more tips in its frequency-tuning curve (i.e., W- or complex-shaped frequency-tuning curve), a Q10 was calculated only when the threshold at one tip was lower than those at all the other tips by ≥10 dB. The Q10 was calculated based on the tip with the lowest threshold.

To study binaural responses in a VNLL neuron, we created an interaural-level difference curve by plotting the number of spikes against the difference in sound pressure level between the ipsilateral and contralateral ears. The degree of binaural interaction was evaluated by measuring the percentage reduction/increase in the number of spikes produced by a dichotic tone burst relative to that produced by the contralateral sound alone. An EI response was defined as one in which the monaural contralateral response was reduced by >20% by simultaneous presentation of an ipsilateral stimulus. An EE response was one in which the contralateral response was increased by >20% by simultaneous presentation of an ipsilateral stimulus. An EO response was one in which the contralateral response was neither increased nor decreased by as much as 20% with simultaneous ipsilateral stimulation.

The effect of NBQX on VNLL neurons was evaluated by comparing the number of spikes elicited by both contralateral and dichotic tone bursts recorded before and during drug application.

**RESULTS**

We recorded from a total of 99 neurons and examined their responses to monaural tone bursts presented to the contralateral ear. In 96 of the neurons, we also examined the responses to dichotic tone bursts. The effects of the neurotransmitter receptor antagonist NBQX were examined in 17 of 96 neurons.

**Spontaneous activity**

The level of spontaneous activity was typically low for VNLL neurons. Nineteen of the 99 neurons showed no spontaneous firing and most of the remaining neurons had very low spontaneous firing rates. Spontaneous activity was <30 spikes/s in 90% of the neurons recorded. The median spontaneous firing rate for the entire sample of 99 neurons was 2.3 spikes/s and the mean was 11.2 spikes/s.
Responses to monaural tone bursts: temporal firing patterns

In each of the 99 neurons, we examined the PSTHs of spikes evoked by 100-ms tone bursts, 20 dB above the cell’s threshold at CF. Figure 1 shows various PSTH patterns encountered in VNLL together with the number of neurons that fell into each category. The response patterns fell into two main groups: onset and sustained. Onset responses were defined as a transient firing of action potentials during the first 30 ms after the onset of a tone, with no additional sound evoked activity for the remaining 100 ms of the tone. These responses were grouped into four categories: phasic, in which only one or two action potentials occurred after the onset of a tone; phasic burst, in which several action potentials were evoked after the onset of the tone; phasic burst with high spontaneous activity, in which a transient onset response was followed by a clear suppression of activity against a background of spontaneous discharges; and double burst, in which action potentials occurred in pairs with a constant interval between the discharges. Sustained responses were defined as a more-or-less continuous discharge of spikes for the duration of the tone. These included four PSTH categories: primary-like, in which the neuron fired in a continuous fashion resembling the responses recorded from 8th nerve neurons; pauser (also called onset-pause-sustained), in which a transient response was separated from continuous firing by a brief pause; irregular sustained, in which the neuron fired in a choppy, irregular pattern for the duration of the tone; and late response, in which the neuron fired after a pronounced delay with continued firing until the end of the stimulus. We also encountered neurons with a fast adapting response pattern, in which activity lasted for >50 ms (half the duration of the tone), but was not sustained for the duration of the stimulus. Although an argument might be made for classifying these as “onset” responses, we decided to consider them as a special group and excluded them from any further analysis of onset versus sustained responses.

We examined the effect of changing stimulus intensity on the shape of the PSTH in 99 neurons. For 78 of these neurons, a change in stimulus intensity did not substantially change the shape of the PSTH, although it increased or decreased the number of spikes. For the other 21 neurons, a change in stimulus intensity resulted in an alteration in the shape of the PSTH. The most frequently encountered result was a change from primary-like at low stimulus intensities to nonprimary-like (e.g., a phasic burst) at high intensities (n = 11). Another pattern was a shift from onset firing at low stimulus intensities to primary-like firing at high intensities (n = 2). Various other changes were found in the remaining 8 neurons.

For each of the 99 neurons, we calculated the mean first-spike latency based on responses elicited by 20 presentations of a tone burst at CF. At 20 dB above threshold at CF, the mean first-spike latencies ranged from 3.1 to 69.5 ms. Considerable variability in first-spike latency was apparent across all sound frequencies. There was no significant correlation between the first-spike latency and the CF of the neuron. For the subset of onset neurons, the first-spike latencies ranged from 3.1 to 31.8 ms (mean = 8.3; median = 7.3) (see Fig. 2 and Table 1). For sustained neurons, the mean first-spike latencies ranged from 5.5 to 69.5 ms (mean = 14.3; median = 9.5). The distributions of first-spike latencies for these two groups of neurons were statistically different (Kolmogorov–Smirnov test, P < 0.02).

For most neurons, the mean first-spike latency decreased as the stimulus intensity increased (Fig. 2, right). As the sound pressure increased from 20 to 50 dB, the mean latencies were reduced by 26 and 29% on average for onset and sustained neurons, respectively. However, there were substantial differences among neurons in the extent of the latency shift with intensity. Some neurons showed either no change or an increase rather than a decrease in latency as the intensity increased. In general, onset neurons showed less variability in latency shift than sustained neurons.

The time of occurrence of the first spike varied from one presentation of a tone to the next. This variability is referred to as the “jitter” in first-spike latency. The SD of the latencies of responses elicited by 20 presentations of a tone burst was calculated to indicate the degree of jitter. For our sample of 99 neurons, the SDs ranged from 0.05 to 3.1 ms with a mean of 0.6 ms and a median of 0.4 ms (Fig. 3A, left). In contrast, the 55 neurons with sustained responses had SDs that ranged from 0.10 to 28.3 ms with a mean of 4.0 ms and a median of 1.2 ms (Fig. 3B, left).
The magnitude of jitter was statistically different for onset and sustained neurons with the stimulus level at 20 dB above threshold (Kolmogorov–Smirnov test, P < 0.001).

We examined the effect of changing stimulus intensity on the degree of jitter (Fig. 3, A and B, right). For neurons with onset responses, the average degree of jitter (i.e., the mean of SDs of the first-spike latency) was essentially constant as the stimulus intensity was increased from 20 to 50 dB above threshold at CF (Fig. 3 A, right). For neurons with sustained responses, however, the degree of jitter decreased by more than 57% when the stimulus intensity was increased by the same amount (Fig. 3 B, right).

Responses to monaural tone bursts: frequency-tuning characteristics

The CF was determined for each neuron in which a single best frequency (lowest threshold) could be determined in its tuning curve. The CFs were broadly distributed from 1.5 to 40 kHz with thresholds ranging from −10 to 75 dB SPL. The CFs and thresholds at CF are plotted in Fig. 4 together with the rat’s behaviorally determined auditory sensitivity curve (Kelly and Masterton 1977). The physiological thresholds for individual neurons covered a wide range of sound pressure levels at each sound frequency.

Frequency-tuning curves were determined for 87 neurons. A variety of tuning curves were found, with V- or U-shaped curves being the most common (Fig. 5, A and B). Together these groups accounted for 55% of our sample (48 of 87 cells). Six neurons with asymmetrical V-shaped or enclosed tuning curves were also found (Fig. 5, C and D). Multipeaked tuning curves, including W-shaped (Fig. 5 E) and other complex shapes (Fig. 5 F), accounted for 37% of the sample (33 out of 87 cells). The number of neurons in each of these categories is indicated beside the corresponding tuning curves shown in Fig. 5.
Q₁₀ values were determined as a measure of the narrowness of frequency tuning in 70 neurons as indicated in Fig. 6A. The Q₁₀ values ranged between 1 and 39.5 with a mean of 7.1. Figure 6B shows that the Q₁₀ values were positively correlated with the CFs of VNLL neurons (r = 0.56). The mean bandwidth of tuning curves was nearly constant across frequency for CFs in the range from 5 to 30 kHz (mean = 2.94 kHz; SD = 2.01). At lower frequencies the tuning curve bandwidths were somewhat smaller (mean = 1.19; SD = 0.41), perhaps as a result of the rat’s limited sensitivity to tones <5 kHz (see Fig. 4).

Responses to dichotic tone bursts

Responses to dichotic tone bursts were examined in 96 neurons. As illustrated in Fig. 7, A–D, responses included no interaction (EO), binaural suppression (EI), binaural summation (EE), or a combination of binaural summation and suppression (EE/EI). Fifty-eight neurons in our sample (60.4%) displayed simple EI interactions, with the maximum binaural suppression ranging from 20 to 100% (Fig. 7B). Thirteen neurons showed EE interactions (Fig. 7C) and 13 neurons showed combined EE/EI interactions (Fig. 7D). The remaining 12 neurons showed no binaural interaction. Binaural responses were distributed across all electrode penetrations and along the entire dorsal to ventral extent of VNLL. There was no indication of a spatial segregation of monaural and binaural responses. The strength of binaural suppression or summation is indicated for all neurons in Fig. 7, E and F.

Response characteristics and location of the recording site

We examined whether the temporal, spectral, or binaural response characteristics were dependent on the location of the neurons along the dorsoventral extent of VNLL (Fig. 8, A–D). As can be seen in Fig. 8A, there was no correlation between CF and dorsoventral position (r < 0.001). Also, there was no relation between position and first-spike latency (r = 0.014) or strength of binaural suppression (r = 0.059) (Fig. 8, B and C). Furthermore, there were no significant correlations between position and either Q₁₀ or the degree of jitter in first-spike latency (see Table 2 for a summary of all correlations).
Effect of blocking AMPA receptors

The effect of NBQX on responses to contralateral and dichotic tone bursts was determined in 17 neurons. Two examples are shown in Fig. 9, A and B. Before the drug, the neuron in Fig. 9A had a monotonic rate-level function in response to monaural tone bursts and an interaural-level difference curve showing an EI interaction in response to dichotic tone bursts. The responses to both monaural and binaural stimuli were reduced substantially during the drug. The neuron in Fig. 9B displayed a nonmonotonic rate-level function and an interaural-level difference curve similar to that of the neuron in Fig. 9A. During the drug, the responses were considerably reduced under all stimulus conditions.

The percentage of reduction in firing to a contralateral tone burst for each of the neurons tested with NBQX is shown in Fig. 9C. The drug resulted in a reduction in response regardless of the strength of binaural suppression in the cell being tested. Both monaural and binaural neurons were affected by application of NBQX as indicated in Fig. 9D.

### DISCUSSION

**Temporal response patterns**

The temporal pattern of firing by neurons in response to tone burst stimulation is an important reflection of the way in which auditory information is conveyed and processed in the CNS. Neurons in the rat's VNLL exhibit a diversity of temporal firing patterns similar to those reported for other mammals. Many neurons (36%) had onset responses including phasic, phasic burst, and double-burst patterns. Other neurons (55%) had sustained responses including primary-like, pauser, irregular sustained, and late response patterns. Also, a small number of neurons (6%) exhibited fast adapting responses that could not be classified as either onset or sustained.

Similar response patterns are present in the VNLL/INLL complex (ventral and intermediate nuclei of the lateral lemniscus) of the big brown bat (*Eptesicus fuscus*) (Covey and Casseday 1991). Within the complex as a whole, 38% of the neurons had onset or phasic responses and 62% had sustained responses that were further classified into primary-like, tonic, chopper, and pauser patterns. The proportion of onset and sustained responses in the bat’s VNLL was similar to that found in rats. However, unlike the rat, the big brown bat had an uneven distribution of response types within the VNLL/INLL.
In particular, the responses in the columnar subdivision of the bat’s VNLL were almost all of the phasic type (95% of the neurons sampled from that subdivision), whereas the responses in INLL and the multipolar subdivision of VNLL had a more even distribution with regard to their temporal response patterns.

Both onset and sustained firing patterns were found in the VNLL/INLL complex of the horseshoe bat (Rhinolophus rouxi) (Metzner and Radtke-Schuller 1987). These response types were represented in approximately equal numbers, although the exact percentage is difficult to compare with other species because of differences in the way in which response types were classified. The proportion of cells with onset and sustained responses was different for INLL and VNLL. The VNLL had a higher percentage of cells with sustained responses.

Batra and Fitzpatrick (1999) found that 49% of the cells in the rabbit’s VNLL had phasic (i.e., onset) responses and 42% had sustained discharges. Sustained responses included pauser, chopper, and irregular patterns. A small percentage of the phasic cells (<10%) in the rabbit’s VNLL were classified as “transient,” having responses that showed strong adaptation to tone bursts. This response pattern resembles our “fast adapting” category.

In the cat, Aitkin et al. (1970) found both onset and sustained responses. The number of cells sampled was small (n = 17) and no breakdown was given of the proportion of cells in the two categories. Guinan et al. (1972a,b) reported that 17 out of 25 neurons in the cat’s VNLL had sustained responses including primary-like, pauser, and chopper patterns. Six neurons had onset or phasic responses, one neuron had a long latency response, and one neuron showed a reduction in firing during tonal stimulation.

It should be mentioned that the classification of the temporal response pattern for some neurons depends on stimulus conditions. In the horseshoe bat 17% of the neurons changed their response class depending on the frequency or intensity of the stimulus (Metzner and Radtke-Schuller 1987). A level-dependent change in response category was also apparent in about 20% of the neurons in the rat’s VNLL.

All mammals examined to date show a diversity of temporal response types in the VNLL or the VNLL/INLL complex. The relative proportion of onset to sustained cells is roughly the same across species, but the distribution of these cell types within the VNLL/INLL is species dependent.

**Precision of timing of the responses in VNLL**

It has been suggested that the neurons in the VNLL are particularly well suited for preserving and processing temporal information in the auditory system (Oertel and Wickesberg 2002). In the rat’s VNLL many neurons exhibit precision in the timing of their responses, but these neurons are widely distributed throughout the nucleus and do not form a spatially segregated population as they do in the VNLL of the big brown bat (Covey and Casseday 1991).

The first-spike latencies for neurons in the rat’s VNLL cover a wide range with average values longer than those in the rat’s cochlear nucleus (Møller 1975) or lateral superior olivary nucleus (Finlayson and Caspary 1993; Irvine et al. 2001), but shorter than those in inferior colliculus (Palombi and Caspary 1996). In the VNLL, neurons with onset responses had shorter mean first-spike latencies than those with sustained responses (8.3 and 14.8 ms, respectively). There were fewer long-latency responses among neurons with onset responses. There was generally a decrease in latency with increased intensity for both onset and sustained cells, but there was less variability in the relation between latency and intensity among onset neurons. These data from VNLL can be compared with the narrower range of response latencies (3.8 – 6.2 ms) recorded from neurons in the rat’s lateral superior olive (Finlayson and Caspary 1993).

Generally, onset cells had less jitter in their first-spike latency than neurons with sustained responses. The variability in the first-spike latency for onset cells was very small with a mean SD of 0.59 ms and a range in SDs from 0.053 to 3.1 ms. Neurons with sustained responses showed less precision with a mean SD of 4.0 ms and a range from 0.10 to 28.3 ms. Furthermore, the jitter in first-spike latency for onset cells changed very little with an increase in sound pressure level, whereas that of sustained cells decreased substantially with sound pressure level. Therefore the onset cell population in the rat’s VNLL could preserve temporal information in spite of changes in stimulus intensity.

Neurons with a high degree of temporal precision in their response have been reported for the columnar region of the VNLL in the big brown bat (Covey and Casseday 1991). In this specialized region of the bat’s VNLL the degree of jitter was even less than that found in the rat’s VNLL. The SD of first-spike latencies for onset neurons in the rat’s VNLL could be as small as 0.03 ms. The mean value for neurons in the columnar region was 0.14 and the range was 0.03 to 0.78 ms. The high degree of temporal precision of onset responses in the big brown bat’s VNLL is probably related to the structural differentiation of the nucleus and its functional specialization for echolocation.

**Intrinsic and synaptic basis of temporal response characteristics**

Brain slice studies have revealed various temporal patterns of response to depolarizing intracellular current injections in the rat’s VNLL (Irfan et al. 2005; Wu 1999; Zhao and Wu 2001). Some neurons (type I cells) respond to steady depolarization with sustained discharge patterns, which can be further classified as regular, onset-pause, or adapting. These three intrinsic response patterns resemble the chopper, pauser, or primary-like patterns recorded from VNLL in vivo. Other neurons (type II cells) respond to depolarizing current injections with a single spike or a few spikes at the beginning of each current injection. These responses are similar to onset responses to tone bursts recorded from VNLL in vivo.

Some response types recorded in vitro change their temporal pattern as the level of membrane depolarization is shifted. Wu (1999) reported that some onset-pause patterns can be changed to sustained patterns by holding the membrane potential at a more depolarized level. This type of change resembles the level dependency of responses in the “mixed” category reported for neurons in VNLL of the horseshoe bat (Metzner and Radtke-Schuller 1987) and the response of many neurons encountered in the present study of the rat’s VNLL. Thus intrinsic membrane characteristics could account at least in part...
for the firing patterns recorded in vivo from neurons in the VNLL.

The responses of neurons in the VNLL are also dependent on synaptic inputs arriving from afferent projections as well as local circuitry within the lateral lemniscus. The VNLL receives direct projections primarily from the contralateral VCN and, to a lesser extent, from the ipsilateral MNTB. In addition, there are ipsilateral projections from the lateral nucleus of the trapezoid body (LNTB) and the ventral nucleus of the trapezoid body (VNTB) (Adams 1997; Friauf and Ostwald 1988; Glendenning et al. 1981; Schofield and Cant 1997; Sunej et al. 1995). Excitatory projections from the VCN would be expected to impose their response patterns on target neurons in the VNLL. It is known from intracellular in vivo recordings that neurons in the anteroventral cochlear nucleus (AVCN) have primary-like or chopper response patterns and that these patterns are associated with bushy or stellate cells, respectively (Rhode et al. 1983). Both of these cell types project to neurons in the VNLL and could provide the excitatory drive to determine response properties of postsynaptic neurons (Friauf and Ostwald 1988; Schofield and Cant 1997; Thompson 1998).

Also, the octopus cells in the posteroventral cochlear nucleus (PVCN) have onset response patterns and project directly to neurons in VNLL. This projection could contribute to the onset or transient responses recorded from VNLL neurons.

Brain slice studies have shown that neurons in the rat’s VNLL receive inhibitory inputs from afferent fibers in the lateral lemniscus. Inhibitory postsynaptic potentials evoked by current pulse stimulation of lemniscal fibers are mediated primarily by glycine receptors, but in some cases by γ-aminobutyric acid type A (GABA_A) receptors or both glycine and GABA_A receptors (Irfan et al. 2005). The most likely source of glycinergic inhibition is the MNTB, whose neurons have been shown by immunocytochemistry to contain glycine (Helfert and Aschoff 1997; Helfert et al. 1989; Vater et al. 1992). The less prominent GABAergic inhibition might originate from LNTB or VNTB, whose neurons label positively for GABA or a combination of GABA and glycine (Helfert et al. 1989). The time course for activation of these inhibitory responses is similar to that for AMPA receptor–mediated excitation in VNLL. Therefore it is likely that excitatory and inhibitory synapses converge on VNLL neurons and interact to control and regulate temporal response patterns.

Another source of inhibition emerges from the local circuitry within the VNLL itself. Neurons in VNLL show positive label for glycine, GABA, or a combination of these transmitters (Oliver and Bishop 1998; Riquelme et al. 2001; Saint Marie et al. 1997; Vater et al. 1997; Winer et al. 1995). In addition to their ascending axonal projections to auditory midbrain structures, such as the central nucleus of the inferior colliculus, these neurons have collateral projections that synapse locally within the VNLL (Zhao and Wu 2001). Intracellular in vivo recordings from the rat’s VNLL have shown the presence of an early inhibition that likely arises from collaterals of VNLL neurons activated by excitatory inputs from octopus cells in the contralateral VCN (Nayagam et al. 2005). This early inhibition could serve to control the precise timing of onset responses in VNLL.

Two response types in the rat’s VNLL, the fast adapting and the late response, are not easily attributed to either intrinsic membrane properties or excitatory input from the cochlear nucleus. These responses, which account for 12% of our sample, appear to involve an active inhibitory process during the early or late parts of a response. Whether these or other responses are shaped by glycinergic or GABAergic inhibition in the VNLL must be determined by pharmacological studies of the effects of locally applied synaptic antagonists.

**Frequency-tuning curves**

Neurons in the rat’s VNLL displayed a wide range of CFs and response thresholds covering most of the rat’s audible range (Kelly and Masterton 1977). The most commonly encountered tuning curves were either “V” or “U” shaped (55%) with a single clearly defined CF. Many neurons (37%), however, had multi-peaked tuning curves, suggesting the convergence of input from lower structures in shaping frequency-response properties. Analysis of Q10 values showed that most of the neurons in the rat’s VNLL were narrowly tuned to sound frequency and that the Q10 distribution was similar to that reported previously for the rat’s inferior colliculus or cochlear nucleus (Kelly et al. 1991; Möller 1969, 1972).

Neurons with different CFs were widely distributed along the dorsoventral axis of VNLL. There was no correlation between the dorsoventral position and the frequency response of the neurons. Thus, unlike most of the nuclei within the central auditory system, the VNLL of the rat does not show any simple tonotopic progression along its major structural axis. This result is consistent with anatomical findings that show a wide distribution of retrograde labeling along the dorsoventral length of VNLL after small injections of tracer into frequency-specific regions of the central nucleus of the inferior colliculus (Kelly et al. 1998; Malmierca et al. 1998). The lack of tonotopic gradient is also supported by functional labeling studies using c-fos (Friauf 1992; Glendenning and Hutson 1998; Saint Marie et al. 1999).

Comparative data suggest that there are differences among mammalian species in the tonotopic organization of the VNLL. Aitkin et al. (1970) reported a low-to-high, dorsal-to-ventral tonotopic gradient in the cat’s VNLL, although Guinan et al. (1972b) did not find such a frequency gradient. Covey and Casseday (1991) found a low-to-high, dorsal-to-ventral tonotopic gradient within the columnar division of VNLL and a somewhat less regular tonotopic organization in the INLL and multipolar division of VNLL in the big brown bat. Metzner and Radtke-Schuller (1987) found a high-to-low, medial-to-lateral tonotopic arrangement in the INLL of the horseshoe bat, but did not find any tonotopic organization in the VNLL (most neurons were devoted to a narrow range of frequencies related to the bat’s echolocation signal). We cannot rule out all forms of tonotopic organization in the rat’s VNLL, such as the concentric or helical pattern suggested by Merchán and Berbel (1996) and Merchán et al. (1997), although our data suggest that there are considerable differences among mammals in the tonotopic organization of VNLL and that no obvious dorsal-ventral gradient exists in rat.

**Binaural response characteristics**

The present study shows that a substantial proportion of the neurons in rat’s VNLL are binaural with the predominant response pattern being an EI interaction similar to that found in
the rat’s ICC (Kelly et al. 1991). The binaural responses are distributed along the entire dorsal to ventral axis of the VNLL. Binaural responses have also been found in the VNLL of cats and rabbits. In the cat, Guinan et al. (1972a,b) reported that over half (56%) of the neurons in VNLL were binaural. Aitkin et al. (1970) found a smaller percentage of binaural responses (11%) in cat’s VNLL. In the rabbit, many neurons in VNLL responded to binaural stimulation (Batra and Fitzpatrick 1997, 1999, 2002) with monaural cells concentrated laterally and binaural cells more medially.

In contrast, Covey and Casseday (1991) found that very few of the neurons in the VNLL/INLL complex of the big brown bat had binaural responses. All of the neurons in the columnar and multipolar regions of VNLL in this species were monaural and only a small percentage of cells in the INLL were binaural. Binaural responses were found in a separate nucleus located medial to the VNLL within the lateral tegmentum and in DNLL, but not in VNLL (Covey 1993). Thus the available data suggest that there are significant differences among mammalian species in the presence and proportion of binaural responses in VNLL.

The binaural responses in the rat’s VNLL do not likely arise from binaural interactions originating in the superior olivary complex. First, tract-tracing studies show that the rat’s VNLL does not receive direct projections from the main binaural olivary nucleus, the lateral and medial superior olives (LSO, MSO), or the superior paraolivary nucleus (SPN) (van Adel 1998). Second, local application of the excitatory amino acid antagonist NBQX rapidly reduces or eliminates both monaural and binaural responses in VNLL, indicating that the responses depend on synaptic excitation within the VNLL, and not on fibers of passage originating from binaural cells in the superior olive.

It is possible that some binaural responses are created from synaptic interactions that occur within the VNLL. The VNLL receives its primary afferent projection from the contralateral VCN, an input that is considered to be mainly excitatory. Direct inhibitory projections to VNLL arise from GABAergic and glycinergic neurons in the ipsilateral nuclei of the trapezoid body, MNTB, VNTB, and LNTB (Schwartz 1992). The neurons in the MNTB and VNTB are driven by contralateral acoustic stimulation and are thus not a likely source of binaural interaction (EI responses). On the other hand, those in the LNTB are driven by ipsilateral stimulation and could contribute to the EI binaural interactions found in VNLL.

Another possible source of binaural responses in the VNLL is the cochlear nucleus. Although the VCN is usually considered to be a monaural nucleus, recent evidence indicates that its neurons are significantly affected by binaural input. First, anatomical studies have shown that there are direct connections between the cochlear nuclei on the two sides of the brain (Alibardi 1998; Cant and Gaston 1982; Schofield and Cant 1996; Shore et al. 1992). Commissural projections originate from multipolar cells and make synaptic contact with the major cell types of the contralateral VCN, including multipolar cells, bushy cells, and octopus cells. Immunocytochemical studies indicate that the multipolar cells that give rise to the crossed projections between the two cochlear nuclei are glycinergic and/or GABAergic and are thus a likely source of binaural inhibition (Alibardi 1998, 2000).

Physiological studies also support the idea of binaural interactions occurring in the VCN. Binaural inhibitory responses have been recorded from chinchilla VCN neurons (Recio 2005). Furthermore, isolated whole brain preparations in the guinea pig have revealed that electrical stimulation of the auditory nerve can elicit inhibitory postsynaptic potentials in bushy and stellate cells in the contralateral VCN (Babalian et al. 2002). In vivo intracellular recordings from rats have shown that stellate cells in the VCN receive monosynaptic inhibitory commissural inputs from cells in the contralateral VCN (Needham and Paolini 2003). In guinea pigs physiological recordings with multichannel probes have shown that spontaneous firing in VCN neurons can be suppressed by contralateral acoustic stimulation (Shore et al. 2003). Thus the binaural responses in the rat’s VNLL might reflect inhibitory/excitatory interactions that occur first within the VCN.

In conclusion, most of the neurons in the rat’s VNLL are clearly tuned to sound frequency with V- or U-shaped tuning curves, although many cells have more complex, multipeaked curves. Temporal responses to tone bursts include both onset and sustained firing patterns. Many neurons in VNLL, particularly those with onset responses, show a high degree of temporal precision in their first-spike latencies, suggesting a role in temporal processing. A large percentage of cells in VNLL are binaural and exhibit EI binaural interaction patterns. However, further studies with selective receptor antagonists are needed to determine whether these binaural responses are created by synaptic interactions locally or are inherited from binaural interactions occurring elsewhere in the central auditory system.

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GRANTS

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