Temporal properties of responses to sound in the ventral nucleus of the lateral lemniscus

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THE VENTRAL NUCLEUS of the lateral lemniscus (VNLL) is an auditory nucleus at a hierarchical level between the cochlear nucleus (CN) and the inferior colliculus (IC). It is embedded in the lateral lemniscus, a large afferent fiber tract to the IC. Because of its poor accessibility, the VNLL is one of the least-studied nuclei in the auditory brain stem, but it shows remarkable features. First, it provides a massive input to the ipsilateral IC (Brunso-Bechtold et al. 1981; Whitley and Henkel 1984). Of the dozen or so inputs to the IC, the VNLL consistently shows the highest counts of retrogradely labeled neurons after IC injections (Cant 2005). Second, it is largely inhibitory: most VNLL neurons are immunoreactive to glycine and/or GABA, which makes the VNLL also the single largest inhibitory source to the IC (Saint Marie et al. 1997; Zhang et al. 1998). Finally, many anatomical and physiological observations suggest that the VNLL is important in various forms of temporal processing (Adams 1997; Covey and Casseday 1991; Guinan et al. 1972a, 1972b; Nayagam et al. 2005, 2006; Oertel and Wickesberg 2002; Pollak et al. 2011; Schofield and Cant 1997).

A few previous studies have drawn attention to temporal properties of the VNLL in echolocating bats, which have a specialized region called the VNLLc. In this region, neurons have a first-spike latency that is rather invariant across trials and stimulus levels and that is assumed to play a role in the encoding of sound onsets (Covey and Casseday 1986, 1991). Recent work in rabbit (Batra and Fitzpatrick 2002a) and rat (Nayagam et al. 2005, 2006; Zhang and Kelly 2006a) shows that responses to pure tones in these animals resemble those found in the bat. Of special interest are responses to sinusoidally amplitude-modulated (SAM) tones (Batra 2006; Zhang and Kelly 2006b), which indicate the existence of neurons that are narrowly tuned to different modulation frequencies. A role of the VNLL in the coding of temporal pitch has been postulated based on 2-deoxyglucose labeling patterns in response to harmonic complexes (Langner 2005). The picture that emerges from the above studies is that the VNLL exerts a massive and well-timed inhibitory control over IC neurons. Envelope coding in the ventral cochlear nucleus (VCN) and IC has been particularly well studied in the cat and shows large but ill-understood differences between these structures (reviewed by Joris et al. 2004). Given the pivotal role of the IC in the ascending auditory system and the strategic placement of the VNLL in the afferent fiber tract to the IC, we seek to characterize cat VNLL responses to stimulus onset and envelope for a broader set of stimuli than used so far. We find narrow modulation tuning as described in other species and examine whether such tuning persists in response to broadband nonperiodic stimulation. In the course of the experiments we also examine basic binaural properties of VNLL neurons.

METHODS

Recordings were obtained from 13 adult cats (3–6 kg) with clear eardrums at the University of Leuven and at the University of Wisconsin-Madison. At both locations, the work conformed to the animal use standards of the National Institutes of Health and was approved by the local animal care committee. In Leuven, cats were anesthetized with an initial dose of ketamine (20 mg/kg im) and acepromazine (0.2 mg/kg im). In Madison, pentobarbital sodium (50 mg/kg ip) was used to induce anesthesia. The rest of the experimental approach was nearly identical for both laboratories. The animal’s rectal temperature was maintained at 37°C with a heating pad. A
surgical level of anesthesia was obtained throughout the duration of the experiment by infusion of pentobarbital through a catheter inserted in the femoral vein, at doses that suppressed the pedal withdrawal reflex. A tracheal cannula was inserted along with a CO2 sensor. A length of polyethylene tubing was inserted into small holes in the exposed bullae in order to keep normal middle ear pressure. Both pinnas were removed to expose the external auditory meatus and allow insertion of ear bars into each ear canal. Modified dynamic speakers (RadioShack super tweeter) mounted in metal canisters were coupled to the ear bars, as well as a probe tube and a Bruel and Kjaer 1/2-in. microphone to enable acoustic calibrations between 0.1 and 40 kHz.

Two surgical approaches were used to record single-unit activity from the VNLL. In the first approach, a craniootomy was made in the skull at a location just anterior to the cat’s bony tentorium. Parts of the occipital cerebral hemisphere were removed to expose the IC. The electrode penetrated the IC at ~5–7 mm from the midline in a parasagittal plane, VNLL recordings were obtained starting at a depth of 5–7 mm from the surface, as judged from dominantly monaural, contralateral responses and, in some cases, a discontinuity in the progression of the units’ characteristic frequency (CF: frequency of lowest threshold) as the electrode leaves the dorsal nucleus of the lateral lemniscus (DNLL), which is a binaural and tonotopically arranged nucleus (Aitkin et al. 1970; Davis et al. 2007). In the second approach, cerebellum was removed until the floor of the 4th ventricle (near the IC) was visualized. The cerebellum overlying the IC was then removed, and the electrode was aimed at a position ventral to the IC again ~5 mm from the midline. Warm agar was applied over the brain to reduce pulsations.

Glass insulated Pt-Ir electrodes and KCl-filled glass pipettes were used to record neural activity. (Seventy-six percent of the recordings were obtained with the Pt-Ir electrodes.) When we used metal electrodes, small currents were applied (10 μA, 10 s) at the electrode tip to mark locations in the tissue, enabling reconstruction of the site of the recordings. We typically performed multiple penetrations in each animal: lesions were made in at least two penetrations per experiment. To maximize the likelihood of recording from VNLL neurons, we kept the position of the animal’s head and the position of the electrode approximately constant across experiments. None of the VNLL neurons presented here had long latencies or was excited by ipsilateral sounds. These physiological properties are characteristic of cell groups in the vicinity of the VNLL, such as the cat’s dorsomedial periolivary nucleus and most neurons that surround the lateral superior olive complex (Guinan et al. 1972a, 1972b).

Cats were perfused through the heart at the end of 8 of 13 experiments, first with 1 liter of saline followed by 1 liter of 10% formalin. Blocks of brain tissue were frozen and serially sectioned at 60 μm. Every section was mounted and stained with cresyl violet, and penetrations were reconstructed based on projections of the histological slides and the presence of lesions. For the animals for which histology was available, the sample reported here was judged to be within the nuclear boundaries of the VNLL on the basis of the Nissl stains and lesions.

The VNLL is not one homogeneous nucleus and has been termed the ventral complex of the lateral lemniscus by some authors (Malmierca et al. 1998). On the basis of Nissl staining, the cat’s VNLL can be subdivided into ventral, middle, and dorsal sections (Adachi-Usami 1979). The dorsal division has also been called the intermediate or interstitial nucleus (INLL) by other authors (Glenddenning et al. 1981), who consider it a transition zone between the DNLL and the rest of the VNLL. In this work we consider the INLL as part of the VNLL.

Hardware and software. In both laboratories, RadioShack super tweeter phones were connected with Tygon tubing to Delrin (Leuven) or metal (Madison) earpines that fit tightly in the cut ear canals. Synthesis of sound stimuli and timing of neural activity were achieved by dedicated hardware and software. In Leuven, custom software, run with MATLAB (MathWorks, Natick, MA) on a personal computer, was used to produce the stimuli and control the digital hardware (Tucker-Davis Technologies, Alachua, FL). In Madison, stimuli were generated by a digital stimulus system (Rhode 1976) controlled by a VAX minicomputer. Stimuli consisted of tone bursts, tones that were amplitude modulated by tones or by narrowband noise, and broadband pseudorandom noise. Neural signals were amplified and filtered (300–3,000 Hz) with a differential amplifier (DAM 80, World Precision Instruments, Sarasota, FL) and converted to pulses with an oscilloscope. The pulses were subsequently time stamped at 1-μs resolution by dedicated hardware (Leuven: ET-1, Tucker-Davis Technologies; Madison: custom-made event time recorder).

Acoustic stimuli. After isolation of single-unit activity, a threshold tuning curve was obtained to contralateral tone bursts with an adaptive tracking algorithm. The tuning curve yielded the CF, spontaneous activity (measured over ~15 s of silence), lowest threshold, and bandwidth (measured at 10 dB above the lowest threshold). Subsequently, contralateral tone bursts of short duration (typically 25 ms every 100 ms or 50–60 ms every 250 ms; 200 repetitions) were presented either at the neuron’s CF at a supra-threshold level of 20–30 dB or sometimes over a large range of SPLs, for the purpose of classifying the poststimulus time histogram (PSTH). In some cases, a response area was obtained by stimulating at many frequency-SPL combinations, for the purpose of estimating the frequency tuning and temporal response patterns of the neurons.

Amplitude-modulated (AM) signals were constructed by multiplying the sinusoidal carrier, sin(2πft), by the envelope, n(t), i.e., s(t) = [1 + m·n(t)]·sin(2πft). The frequency of the carrier was always equal to the neuron’s CF. Two types of AM were used. In sinusoidal AM (SAM), the envelope was a sinusoid of frequency fmod, i.e., n(t) = sin(2πfmod·t). In noise AM (NAM), the envelope n(t) was a sample of a standard normal (Gaussian) distribution generated with the MATLAB function randn and low-pass filtered with a 1-kHz cutoff frequency. The modulation depth m for SAM was always equal to 1 (100% modulation), i.e., the carrier amplitude was 6 dB above the amplitude of the two spectral sidebands. For NAM, the modulation depth was set at 50% to limit overmodulation. The duration of the SAM and NAM stimuli was usually 400 ms, presented 10 and 100 times, respectively, once per second.

Finally, pseudorandom broadband Gaussian noise (50-kHz bandwidth) was also presented. Again, this stimulus was generated with the MATLAB function randn. The duration of the broadband stimuli was 200 ms, presented 5 times every second, and the overall level was usually 50 or 70 dB. SAM, NAM, and broadband noise stimuli were applied to the contralateral ear. Rate-intensity functions (RIFs) were obtained with either 100-ms tones or 100-ms broadband noise over an 80–90 dB range. Stimuli used for RIFs were usually presented 10 times every 250 ms.

We studied sensitivity to interaural level differences (ILDs) by presenting contralateral pure tones at CF at a fixed level, 20–40 dB re threshold, while randomly varying the level of an ipsilateral tone at the same frequency between 0 and 80 dB SPL. Each stimulus combination was presented 10 times with a duration of 200 ms and a repetition interval of 1 s.

Data analysis. We quantified sharpness from the threshold tuning curve with the traditionally used quality factor Q10 (CF/bandwidth) or by narrowband noise, and broadband pseudorandom noise. Neural signals were amplified and filtered (300–3,000 Hz) with a differential amplifier (DAM 80, World Precision Instruments, Sarasota, FL) and converted to pulses with an oscilloscope. The pulses were subsequently time stamped at 1-μs resolution by dedicated hardware (Leuven: ET-1, Tucker-Davis Technologies; Madison: custom-made event time recorder).
50 ms after stimulus onset. tMTF values are only plotted and analyzed if the SCs are statistically significant ($P < 0.01$, Raleigh test; Mardia 1972). Bandwidths of MTF functions were defined as the frequency range within which the rate (or synchrony) is above half its maximum value.

Responses to NAM were analyzed by using a modification of the reverse correlation technique (de Boer 1967) to derive the average stimulus preceding a spike (the prevent stimulus ensemble, PESE) (Johannesma 1972). Rather than using the entire stimulus waveform, we use the envelope, $n(t)$, to compute the “PESE envelope” (Keller and Takahashi 2000). The envelope $n(t)$ was computed as described in Acoustic stimuli.

Responses to broadband noise were used to estimate spectrotomoral receptive fields (STRFs) (Aertsen and Johannesma 1981). These were obtained by filtering the broadband noise stimulus with a bank of 50 band-pass (gammatone) filters and computing the envelope at the output of each filter to estimate within-channel PESEs. For this analysis, the envelope was computed with the Hilbert transform of the output of each filter (Recio-Spinoso et al. 2009). The best frequencies of the gammatone filters were equally spaced in a logarithmic axis between 0.1 and 25 kHz.

Data from auditory nerve fibers and lateral superior olive. Mean and standard deviation of first-spike latencies, measured in the responses of auditory nerve fibers (ANFs) to single tones, were compared to the same measures obtained from responses of VNLL neurons. We used archival ANF data, previously collected in Madison (see Figs. 3 and 4). We also compared binaural sensitivity of VNLL neurons to that of neurons in the lateral superior olive (LSO), also collected in Madison (see Fig. 16).

RESULTS

From the 307 neurons recorded, we classified 148 as originating in the VNLL. This was based on histological reconstruction in 8 of the 13 experiments, depth of penetration, and two physiological criteria: a discontinuity in the tonotopic progression from the structures dorsal to the VNLL (e.g., IC and DNLL) and predominance of monaural contralateral responsiveness (see METHODS). Eighty percent of the putative VNLL neurons in this study came from the cats whose brains were processed histologically. These recordings were obtained after gaining access to the VNLL via the first and second surgical approaches (see METHODS) in four and five cats, respectively. (In 1 of these cats, the VNLL was accessed with the 2 surgical approaches.) We first provide evidence for the existence of two basic classes of discharge patterns, based on the responses to pure tones. We then illustrate that these two groups also differ in their response to AM stimuli and broadband noise.

Responses to single tones. Examination of the PSTHs constructed from response of VNLL neurons to single tones showed that two broad classes of PSTH patterns could be distinguished: onset and sustained. Neurons with an onset response fire action potentials nearly exclusively at stimulus onset. Conversely, neurons with a sustained response fire action potentials throughout the entire stimulation period. Spontaneous rate (SR) was low in both classes of neurons. SR mean values were 1.03 and 7.46 spikes/s for onset and sustained neurons, respectively. Differences between SR mean values were statistically significant ($t$-test, $P = 0.0163$). These two discharge patterns were also observed by Aitkin et al. (1970) in their VNLL recordings in the cat. Instead of relying on visual examination of the PSTH to classify a cell, we used the quantitative criteria developed by Batra and Fitzpatrick (1999). In this scheme, an “onset-response” neuron has a steady-state (>35 ms after stimulus onset) driven rate below 25 spikes/s with a driven transient (<35 ms) rate at least twice the steady-state rate. Otherwise, the neuron is classified as a “sustained-response” neuron. In those cases in which responses were obtained at multiple SPLs, the PSTH class was consistent across these different stimulus conditions. Further subclassification of PSTH shape is possible. For example, following nomenclature used in the VCN, sustained responses can be further classified as primary-like or chopper, which are indeed also two of the most common PSTHs found in our experiments (Fig. 1). Neurons displaying buildup or on-off PSTHs were also found but less common. Nevertheless, we refrain from further subclassification and limit the classification to the onset and sustained PSTH classes. Thirty-one percent of the total number of VNLL neurons were onset, 58% were sustained, and the remaining 11% did not fit either category (i.e., sustained neurons with latencies > 30 ms).

Representative examples of PSTHs to short tone bursts at CF are shown in Fig. 1. Some onset neurons (Fig. 1A) respond to near-CF tones with only one or two spikes at stimulus onset. This is reminiscent of the response of octopus neurons in the VCN (Godfrey et al. 1975; Rhode et al. 1983; Smith et al. 2005). Other onset neurons respond very reliably to the stimulus onset but also fire a small number of action potentials during the steady-state part of the stimulus (Fig. 1B). Sustained-response neurons display PSTHs with shapes akin to those observed in the VCN, e.g., primary-like (Fig. 1C) or chopper (Fig. 1D). Note that sustained and onset responses are found across the entire CF range (see Fig. 2).

The frequency tuning of VNLL neurons was evaluated with threshold tuning curves. Figure 2 shows examples of threshold tuning curves, with accompanying PSTHs, for onset- and sustained-response neurons. A diversity of tuning curve shapes was present, ranging from narrow V shapes to broad curves including W shapes, as in Fig. 2, A and G. W shapes were observed in ~15% of the total number of cells in our sample. We also observed a corresponding variety of $Q_{10}$ values, with a high of 8.5 in Fig. 2E and lows of 1.48 and 1.76 in Fig. 2, A and C. $Q_{10}$ values of the overall sample of sustained and onset neurons do not show statistically significant differences (Kolmogorov-Smirnov test, $P = 0.0719$). $Q_{10}$ values increased with CF up to ~5 kHz and remained scattered thereafter around an average of 6.26 (Fig. 2I). $Q_{10}$ values are, of course, incomplete descriptors of these tuning curves because of the presence of multiple peaks. In this situation, $Q_{10}$ was evaluated by finding the most sensitive frequency and estimating the 10-dB bandwidth using that frequency as reference.

Figure 2I reveals differences between the frequency tuning of VNLL neurons and ANFs. Despite the large fluctuations in $Q_{10}$ values shown in Fig. 2I, $Q_{10}$ values of ANFs are statistically larger than those computed from VNLL neurons (single-tailed $t$-test, df = 130, $P = 0.0028$).

Neurons in the columnar region of the bat’s VNLL (VNLLc; Covey and Casseday 1991) respond to tones with precise first-spike latency at the stimulus onset, which contrasts with the variability in the responses exhibited by sustained neurons in the same species. Figure 3 displays average first-spike latencies (Fig. 3A) and their standard
deviation $\sigma_{fsl}$ (Fig. 3B) for a sample of VNLL neurons ($n = 148$) in response to 20–30 dB suprathreshold tones at CF. Both onset and sustained neurons show a large range of first-spike latency values. The lower envelope of the distribution parallels the mean first-spike latency distribution in the auditory nerve, shown in Fig. 3A with a solid line for comparison. Interestingly, the variability in first-spike latency ($\sigma_{fsl}$) is smaller for onset than for sustained neurons and ANFs (Fig. 3B): 71% of onset neurons have $\sigma_{fsl} < 1$ ms, compared with 28% of sustained response neurons. The variability in first-spike latency is not correlated with CF, as shown by the square values of the correlation coefficients for sustained and onset neurons (0.02 and 0.05, respectively). Differences in mean values in the two types of data shown in Fig. 3B are statistically significant ($t$-test, $P < 0.0001$).
remarkable type of response in the so-called constant-latency VNLL. However, Covey and Casseday (1991) identified a similar dependence would be expected in the VNLL neurons, located in a specific subregion of this nucleus in the bat, the VNLLc. These neurons have mean first-spike latencies that remain constant within 1 ms across stimulus level (from 10 dB to 40 dB above threshold). Moreover, in these responses the standard deviation of the first-spike latency ($\sigma_{\text{fsl}}$) at these levels is also $<1$ ms. Figure 4, A and B, show mean first-spike latency and $\sigma_{\text{fsl}}$ over a 40-dB range for two VNLL onset neurons, which both had thresholds near 20 dB SPL. Similarly, Fig. 4, C and D, show latencies for two sustained-response neurons. The responses in Fig. 4, A and B, fit the criteria for constant latency (dashed lines show a separation of 1 ms). Neurons in Fig. 4, C and D, satisfy only one of the two constant latency criteria (standard deviations $<1$ ms). Variations in the latencies across stimulus level observed in Fig. 4, C and D, are $<1$ ms but only from 20 to 40 dB above threshold. We consider neurons in Fig. 4, C and D, near constant-latency neurons. For comparison, responses from two ANFs are shown in Fig. 4, E and F. We restrict this comparison to ANFs of low spontaneous rate, because onset neurons have low spontaneous rates and because the occurrence of spontaneous activity hampers the computation of first-spike latency. We have RIFs for seven low-spontaneous ANFs. The standard deviation of the first-spike latency for this sample of ANFs was always $>1$ ms (minimum $\sigma_{\text{fsl}} = 1.277$ ms). The minimum and maximum variations in first-spike latency were 1.46 ms and 4.46 ms, measured from 20 to 40 dB above threshold, respectively. Clearly, both the dependence of first-spike latency on SPL and the $\sigma_{\text{fsl}}$ are much larger in the subset of low-spontaneous ANFs tested than in the VNLL onset- and sustained-response neurons that fulfill the constant latency criteria either completely (e.g., Fig. 4, A and B) or partially (e.g., Fig. 4, C and D). We studied 17 VNLL onset-response and 20 sustained-response neurons with CF tones at multiple levels (between 10 and 40 dB above threshold): in all these neurons latency was remarkably invariant with SPL. Five onset VNLL neurons satisfy both criteria. Three onset and three sustained neurons satisfy only the standard deviation criterion. Among near constant-latency neurons, $\sigma_{\text{fsl}} < 1$ ms, and changes in latencies as a function of SPL have a mean value = 1.76 ms for stimulus levels 10–40 dB re threshold.

Responses to sinusoially amplitude-modulated tones. Spike trains can synchronize to two types of ongoing features of the sound waveform: fine structure and envelope. Our sample of VNLL neurons showed a clear bias toward high CFs (Fig. 3), and we found synchronization to pure tones at CF to be poor. For neurons with CF $< 1.43$ kHz, SC values were usually below 0.5. The largest SC value was 0.76 computed from the responses of an onset neuron to a tone of 860 Hz (= CF). While synchronization to fine structure was unremarkable, synchronization to envelope was often striking. We studied the ability of VNLL neurons to encode fluctuations in sound amplitude by varying $f_{\text{mod}}$ of SAM tones and evaluating both the synchrony to the stimulus envelope (tMTF) and the average firing rate (rMTF). Throughout the rest of this report we keep the labels “onset” and “sustained” for such neurons based on their response to pure tones.

Figure 5 shows the rMTFs for the neurons for which the pure tone PSTHs were displayed in Fig. 1. The SPL was usually 50 or 60 dB, but if time allowed additional rMTFs were collected at other levels in 20-dB steps (e.g., 30 and 50 dB re threshold).
driven the frequency range at half-height (see METHODS). In rMTF was flat. As a simple measure of bandwidth, we com-
cases a band-pass shape (data not shown), but more often the
low-pass shape was present (Fig. 5, most sustained responders were rather featureless. At most a
was found between CF and rBMF.
A function of CF for the onset neuron sample. No correlation
affected by SPL. Figure 6 for rate (rBMF) could be defined. For those cases where the
of their rMTFs is evident, and a best modulation frequency
B sustained responses (Fig. 5).
In contrast to the rMTFs of onset neurons, the rMTFs of
most sustained responders were rather featureless. At most a
low-pass shape was present (Fig. 5, C and D) and in very few
cases a band-pass shape (data not shown), but more often the
rMTF was flat. As a simple measure of bandwidth, we com-
puted the frequency range at half-height (see METHODS). In
those cases where an upper or lower limit was not reached, the
lowest and/or highest $f_{\text{mod}}$ tested was taken as the bandwidth
limit(s), even though in those cases this measurement only
provides a lower bound. Figure 6B shows bandwidth as a
function of CF for the onset neuron sample. No correlation
was found between CF and rBMF.
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Fig. 3. Mean first-spike latencies were similar for sustained and onset categories (A) but differed in their standard deviation ($\sigma_{\text{fsl}}$: B). Values are plotted as a function of the neuron’s CF for a population of VNLL neurons. Each symbol represents 1 neuron. Thick continuous lines in A and B display average data obtained from ANFs in the cat (A. Recio-Spinoso and W. S. Rhode, unpublished observations).

dB for the neuron with CF = 18,950 Hz in Fig. 5C). Many
onset neurons did not show sustained responses to SAM
stimuli (e.g., most neurons in Fig. 5A). This was particularly
true of onset neurons having a PSTH with one large peak,
such as those in Fig. 1A. Other onset neurons, however, gave
sustained responses (Fig. 5B). The band-pass characteristic
of their rMTFs is evident, and a best modulation frequency
for rate (rBMF) could be defined. For those cases where the
rMTF was available at multiple SPLs, the rBMF was barely
affected by SPL. Figure 6A shows a scatterplot of rBMF as
a function of CF for the onset neuron sample. No correlation
was found between CF and rBMF.
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limit(s), even though in those cases this measurement only
provides a lower bound. Figure 6B shows bandwidth as a
function of CF for the onset neuron sample. No correlation
was found between CF and rBMF.
can be as high as 0.9, but clearly this is much more common in onset cells, where the maximum SC values are >0.9 in 64% of the neurons. The difference between the maximum SCs of onset and sustained neurons was statistically significant (t-test, P < 0.0001).

In summary, onset and sustained neurons differ markedly in their response to SAM. Onset neurons showed selective rMTFs and high envelope synchronization: they tend to be tuned in spike rate and all pass in synchronization. Sustained neurons were more variable but generally showed less selectivity in their rMTF functions and less synchronization.

Responses to noise-modulated tones. In Responses to sinusoidally amplitude-modulated tones, the results for SAM stimuli suggest that VNLL neurons can behave as modulation filters, in terms of either rate (onset neurons) or synchrony (sustained neurons). Below, we test this assertion with stimuli that contain multiple envelope frequencies simultaneously in 12 neurons (5 onset, 7 sustained). SAM stimuli are special in the narrow bandwidth and perfect periodicity of their envelope; if the MTF tuning observed to SAM stimuli extended to stimuli with a broadband and nonperiodic envelope, such as in NAM stimuli, the neurons would qualify as genuine modulation filters.

First, we compare the response patterns evoked by NAM stimuli and tones. Figure 8 shows responses for three VNLL neurons to NAM stimuli (Fig. 8, D–F) and to CF tones (Fig. 8, A–C). These stimuli differ significantly in their envelopes: for the NAM stimuli the envelope varies in amplitude, while for tones it remains completely flat except at the stimulus onset. Nevertheless, we observe a basic similarity in the temporal response pattern to both stimuli. The neuron with the high, sustained response shows chopping that is very similar to tones and NAM (Fig. 8, A and D). Likewise, the neuron with the pure onset to tones also lacks a sustained response to NAM (Fig. 8, B and E). For the neuron with an onset response and low sustained rate to tones, the difference in response between the two stimuli is more marked (Fig. 8, C and F), showing an onset response followed by irregular activity during the sustained part in the response to NAM. In general, we found that some neurons with onset responses to tones were not well driven by SAM tones (Fig. 5A), which is also the case for NAM (Fig. 8, A and D). Neurons with sustained responses (Fig. 5, C and D) were well-driven by both tones and SAM stimuli and also by NAM stimulation (Fig. 8, A and D). Onset neurons with some level of sustained response can be driven by SAM stimuli over a certain range of f_mod (Fig. 5A), which is also the case for NAM (Fig. 8, B and E). Neurons with sustained responses (Fig. 5, C and D) were well-driven by both tones and SAM stimuli and also by NAM stimulation (Fig. 8, A and D).

We characterized the temporal pattern of NAM responses by computing PESE envelopes (see METHODS). Examples of PESE envelopes for six sustained-response VNLL neurons...
are shown in Fig. 9 (insets). The PESE shape varies from monopolar to oscillatory. Fourier transformations of these PESE envelopes are displayed in the main panels of Fig. 9 along with the tMTF functions obtained from the responses of the same neurons to SAM stimuli. Note that both of these characterizations depend on the time structure of the train of action potentials fired by the neurons. The maximum value of the Fourier transforms was normalized to the maximum value of the tMTF to SAM stimuli. There is an overall correspondence in the modulation tuning to SAM and to NAM, both in width and in best modulation frequency.

The similarity between tMTFs and Fourier transforms of the PESE envelopes was quantified using the correlation coefficient, $r$, which equals 0.84, 0.75, 0.75, 0.72, 0.87, and 0.65 for the results shown in Fig. 9, A–F, respectively. (For the data in Fig. 9D, $r = 0.72$ was obtained using the tMTF data at a 30 dB SPL level—
data plotted with thinnest line.) Correlation coefficients were computed using modulation frequency values for which tMTFs and PESE Fourier transforms were both defined.

Not surprisingly, the same analysis performed on responses of onset neurons did not reveal the good match between SAM and NAM data. Figure 10 shows the results for four onset neurons in the same format as Fig. 9. For the neurons for which the tMTF to SAM was significant over a wide range of $f_{\text{mod}}$ (Fig. 10, A and C), the shapes of these tMTFs and the PESE Fourier transforms are very different. Whereas tMTF functions to SAM are usually very broad in the frequency domain (Fig. 7, A and B), the MTFs from the narrowband noise stimulation tend to be low pass and thus more limited in their bandwidth. Correlation coefficients for the results shown in Fig. 10, A and C, were $-0.48$ and $0.45$, respectively. For the two onset neurons that showed synchrony to SAM over a limited range of $f_{\text{mod}}$ (Fig. 10, B and D), there is not much basis for comparison between SAM and NAM data.

**VNLL responses to broadband noise.** In Responses to noise-modulated tones, we showed that for sustained-response neurons there is good agreement between MTFs derived from modulation by pure tones (SAM) or noise (NAM) (Fig. 9). In these stimuli, we imposed deterministic envelopes on the carriers (see METHODS). The broadband noise that we use in this section has no envelope, but cochlear band-pass filtering imposes an envelope that is impressed on the discharge patterns of the ANFs (Louage et al. 2004; Recio-Spinoso et al. 2009) and that can be exploited by the CNS, e.g., it generates sensitivity to interaural time differences of broadband noise in high-CF neurons of the IC (Joris 2003). The question we want to pose here is whether the modulation tuning measured with deterministic envelopes (SAM, NAM) applies to these internally generated envelopes.

At a coarse level of analysis, PSTHs obtained from the responses of VNLL neurons to frozen broadband noise can resemble PSTHs obtained from their responses to CF tones, similar to our earlier observations regarding the response patterns to NAM stimuli (Fig. 8). An example of such resemblance is shown in Fig. 11 for four neurons, where each column represents the responses of the same neuron to CF tones (Fig. 11, A–D) and broadband noise (Fig. 11, E–H). Although the histograms in Fig. 11, D and H, exhibit certain differences in the responses to tones and noise—particularly during the steady-state response component—the overall onset shape of the histograms remains. In general, we conclude that the shape of the PSTH computed with CF tones remains similar to the one obtained with frozen noise; in other words, the onset or sustained PSTH shape remains the same. As we will illustrate below, the temporal structure in the steady-state part of the response reflects envelope synchronization to the repeated pseudorandom noise sample.

Onset neurons generally respond to noise more vigorously than to CF tones. Figure 12, A and B, show RIFs for two onset neurons. Broadband noise evokes much larger maximum firing rates than CF tones. Figure 12C compares the firing rates evoked by noise and tones for a sample of VNLL neurons. Although the duration of the noise stimulus was usually 200 ms, firing rates in Fig. 12, A–C, were computed over 50 or 60 ms, relative to stimulus onset, which corresponds to the duration of the tone stimulus.

Because rate-intensity curves, such as those in Fig. 12, A and B, were not routinely measured, data in Fig. 12C represent the
firing rate evoked by a CF tone at (usually) 60 dB SPL plotted against the rate evoked by broadband noise. The broadband noise level was attenuated 20–30 dB relative to the maximum level in our acoustic system. Because the intensity of both stimuli was well above threshold for most neurons, firing rates evoked by those stimuli closely approximate the maximum firing rates. Figure 12C confirms that for onset neurons broadband stimuli evoke larger firing rates than CF tones: 11 of 13 of the data points are below the diagonal of equality. Although noise-evoked rates can be >100 spikes/s, none of the tone-evoked rates is above that value (Fig. 12C). For onset neurons, most rates evoked by tones are <60 spikes/s (Fig. 12C). These firing rate differences are statistically significant (Wilcoxon signed-rank test, $P = 0.003$). In the case of sustained-response neurons, the tendency is in the opposite direction: the response to tones tends to be stronger than to noise (60% of data points...
are above the diagonal). These firing rate differences were also statistically significant, but only after logarithmic transformation (Wilcoxon signed-rank test, $P = 0.04036$). It is also evident from Fig. 12 that the maximum firing rate measured in sustained-response neurons can be much larger (by a factor of 2) than in onset neurons.

STRF analysis (see METHODS) was used to analyze VNLL responses to white noise of 23 neurons. Figure 13A is an example of an STRF computed from the responses to noise of a sustained-response VNLL neuron with a CF = 14.8 kHz (as defined by single tones). The STRF shows a maximum value at a frequency near CF. This analysis was performed in 22 additional neurons, but frequency-tuned STRFs were obtained in only 12 cases (Fig. 13B). For each of these units, the best frequency of the STRF occurred at a frequency that matched the CF of the neuron obtained with pure tones, with the exception of one case (Fig. 13B). These results illustrate that despite the absence of phase-locking to near-CF frequencies it is possible to recover tuning information from the responses of VNLL neurons to white noise.

Comparisons between VNLL responses to broadband noise and AM stimuli. The results shown in Fig. 9 indicate that for sustained-response neurons there exists a good correspondence between MTFs obtained using SAM and NAM sounds. These are both relatively narrowband stimuli. The responses to broadband noise allow us to compare envelope processing to narrow- and broadband stimuli. In working toward that goal, the responses to noise were analyzed by first extracting a “slice” from the STRF plot along the time $x$-axis. The slice corresponds to the maximum STRF value at a frequency near the CF derived from the threshold tuning curve (Fig. 13B). For example, the slice for the response shown in Fig. 13A is displayed in Fig. 14E. The Fourier transform amplitude of that temporal waveform was then computed and compared to MTFs obtained from the responses of the same neurons to SAM and NAM sounds. Waveforms used for this comparison were those that have the largest amplitudes in STRF plots, i.e., those obtained from filters centered at or near CF. (Each waveform was computed as described in METHODS.) Figure 14 shows seven such waveforms taken from the STRFs estimated from the responses of 10 VNLL neurons. Three waveforms (Fig. 14, B, D, and F) originate from responses of onset neurons and the rest from sustained neurons. The waveforms contain oscillations that are characteristic of either low-pass (e.g., Fig. 14A) or band-pass (e.g., Fig. 14G) systems.

A comparison between the reverse-correlation functions derived from STRFs and the tMTFs to SAM stimuli was performed in the frequency domain and is shown in Fig. 15 (the number of neurons, 10, represents the sample size of neurons tested with SAM and broadband noise stimuli). The square root of the Fourier transform amplitude of the PESE envelope was calculated. As expected from the waveforms in Fig. 14, these spectra are low pass (Fig. 15, A, E, and H) or band pass (Fig. 15, B–D, F, G, and I). Also shown are the tMTFs obtained in response to SAM tones at three SPLs (30, 50, and 70 dB SPL; Fig. 15). The Fourier transform ampli-
tude of the PESE envelope is normalized to the maximum value of the SAM responses. Although the two sets of data match poorly for some neurons (e.g., Fig. 15, E–H), overall the similarity of the two sets of data is remarkable as judged from the correlation coefficients, $r$, between the two data sets. Correlation coefficient values in Fig. 15 correspond to the maximum values. ($r$ values for Fig. 15, E–H, are not shown because they are not statistically significant.) Envelope detection is a highly nonlinear process; therefore one would not expect the response to broadband noise to match the sum of the responses to sinusoidal envelope frequencies.

**Binaural properties of VNLL neurons.** Early studies of the VNLL were conflicting regarding the extent of its binaural properties (Aitkin et al. 1970; Guinan et al. 1972a, 1972b). Recent work confirms that sensitivity to ILDs is present in this nucleus in rabbits and rats (Batra and Fitzpatrick 1999; Zhang and Kelly 2006a) but not in bats (Covey and Casseday 1991). We obtained ILD functions by presenting contralateral pure tones at CF at a fixed suprathreshold level 20–40 dB re threshold. The intensity of an ipsilateral tone at the same frequency was then varied randomly between 0 and 80 dB SPL. Positive ILDs refer to a higher stimulus level in the ipsilateral than the contralateral ear. The ILD functions in Fig. 16 display normalized firing rate as a function of the SPL difference (ipsilateral – contralateral) for a sample of 112 VNLL neurons; dashed lines represent data for onset neurons. Clearly, a large proportion of neurons show ILD sensitivity. Ipsilateral sound stimulation evoked decreases in firing rate between 0 and 100%. We contrast this ILD sensitivity with that observed in a sample of neurons in the LSO (Joris and Yin 1995), for which ILD sensitivity is well documented (Boudreau and Tsukitchi 1968) and is shown (with opposite ILD convention) in Fig. 16B. ILD sensitivity differs between the two structures in several respects. First, the sign of the sensitivity is opposite (ipsi ear inhibitory in VNLL and excitatory in LSO). Second, some VNLL neurons display nonmonotonic ILD functions where the effect of the ipsilateral ear is facilitatory at low ipsilateral SPL (large negative ILD) but turns suppressive at high ipsilateral SPL (positive ILD). This is not observed in LSO responses, except in one neuron. Third, at small ILDs (e.g., at 0 ILD) the inhibition is more effective in the LSO than in the VNLL. In particular, VNLL neurons are rarely completely inhibited by ILDs as large as 20 dB, while most LSO neurons are. For all neurons, we calculated the maximal reduction in firing rate by ipsilateral stimulation and express it as a percentage of the response at the most negative ILD (Fig. 16C). In the VNLL, these reductions are usually small (<20% in almost half of the neurons studied), but they are occasionally near maximal. While 80% of LSO neurons had maximum reductions to binaural stimulation of at least 80%, this was the case for only 20% of VNLL neurons. Average ILD thresholds for a 50% reduction in firing rate were ~3.9 and ~13 dB SPL in VNLL and LSO neurons, respectively. Thus, while ILD sensitivity to pure tones is present in the VNLL, it is less prevalent and usually weaker than in the LSO.
DISCUSSION

Among the many inputs to the auditory midbrain, anatomical studies (see introduction) highlight the VNLL as a massive source of inhibition. We characterized a large sample of VNLL neurons with a variety of stimuli to explore the onset and sustained temporal properties of these cells. In agreement with previous studies, we find two broad classes of neurons. Onset neurons stand out for their precisely timed responses to stimulus onsets and envelopes and their sharp rate tuning to envelopes, while sustained neurons stand out for their temporal tuning to envelope components. We show that, particularly for the latter class of neurons, this tuning is quite invariant to the nature of the stimulus (tonal or noise carrier, tonal or noise envelope). The VNLL thus seems well placed to impose time markers or reference signals on different time-scales on the integration of subcollicular inputs in the IC.

Responses to SAM stimuli.

Using SAM stimuli, we obtained responses that show many similarities to the two previous reports in nonecholocating mammals, despite differences in species, techniques, and methods of classification (Batra 2006: awake rabbit; Zhang and Kelly 2006b: anesthetized rat). The predominant rMTF shape of onset neurons is band pass (Fig. 5, A and B), while that of sustained neurons is mostly flat or low pass (Fig. 5, C and D). This is similar particularly to the results in the awake rabbit (Batra 2006) but differs from the results in rats, in which rMTFs of most sustained neurons were also tuned to a certain modulation frequency (Zhang and Kelly 2006b). Note, however, that even in the rabbit some sustained neurons show tuning in their rMTFs (Fig. 4 in Batra 2006).

An important difference between AM responses of VCN and IC neurons is in the shape of the rMTFs computed from such responses: the narrow tuning frequently seen in the IC is rare in the VCN (discussed below; see Responses to single tones) (Joris et al. 2004; Nelson and Carney 2007). Interestingly, the rMTFs in gerbil IC frequently show regions of both rate enhancement and rate suppression, in which the latter can be narrowly tuned with steep slopes (Krishna and Semple 2000). As discussed by those authors, such suppressive regions could originate from rate-tuned inhibitory sources in the brain stem. The present data indicate the VNLL as one of these sources.

Responses to tones modulated by narrowband noise. Brainstem responses to broadband stimuli are sometimes well predicted by summing responses to tones. MTFs are commonly interpreted as modulation “filters,” but do they predict responses to stimuli with complex envelopes? Examining a small sample of CN and IC neurons, Møller (1973) and Møller and Rees (1986) found an excellent match between tMTFs obtained with SAM and with tones modulated by noise. Because of all the implicit nonlinearities in signal demodulation and in neurophysiological processes, this result is not trivial. We used a similar method to examine this issue in the VNLL. Fourier analysis of the cross-correlation between the neuronal output and the narrowband noise modulator yielded a good approximation to the tMTF obtained with SAM, but only in the case of sustained-response neurons (Fig. 9). The match was poor for onset neurons (Fig. 10); moreover, some onset neurons respond to the noise modulation with only a few spikes at the stimulus onset. The poor quality of the match in Fig. 10 might result from saturation in the responses because of the use of 100% modulation depth, and it might have improved with a lower modulation depth.
Responses to broadband noise. Second to pure tones, broadband noise is possibly the most generic stimulus used in auditory research. In addition to being more natural than single tones, this stimulus has the advantage that it can be used with a variety of system identification techniques, such as reverse correlation (de Boer 1967), Wiener kernel analysis (Marmarelis and Marmarelis 1978; Recio-Spinoso et al. 2005), or STRF analysis (Aertsen and Johannesma 1981). In some central auditory nuclei, responses to noise can be predicted to a remarkable extent by summed responses to pure tones (Joris et al. 2005, 2008; Yin et al. 1986), while in other nuclei such predictions fail and reveal nonlinear processing (Nelken and Young 1994; Spirou and Young 1991).

Although there is no envelope as such in broadband noise (except the rectangular gating), narrowband cochlear filtering induces amplitude fluctuations commensurate with the bandwidth of the filter (Joris 2003; Recio-Spinoso et al. 2009; Rice 1945). The question arises of whether the envelope tuning manifest in MTFs to modulated CF tones (either by SAM or narrowband noise) also extends to nonperiodic broadband stimuli for which amplitude modulations arise within the auditory system. Using STRF analysis, we inferred the tuning and best frequency of VNLL neurons that are responsive to noise and, for some neurons, make a reasonable prediction of the shape of the tMTF obtained with SAM. The STRF is a collection of reverse correlation functions: only the function obtained from the output of the filter centered at or around CF was used in the comparison to the tMTF. In general, the square root of the Fourier transform amplitude matched the shape of the tMTF reasonably well for many neurons of both types (Fig. 15).
sample size, especially for onset neurons, we cannot make statements on whether this matching differs between onset and sustained neurons.

Responses to single tones. PSTHs of responses to tones showed a variety of shapes that can be sorted into categories similar to those in the VCN (i.e., primary-like, chopper, onset). We restricted our classification to a simple categorization as onset or sustained (Fig. 1), which also appears to capture trends in terms of envelope coding. Although not dichotomous, several other response properties differ between the two classes, again broadly consistent with those of earlier studies (Batra 2006; Zhang and Kelly 2006b).

Frequency threshold curves range from sharply tuned to wide and complex, with no apparent consistency in terms of onset or sustained category. It is notable that the CF distribution is biased toward high CFs, mostly >4 kHz (e.g., Fig. 3). There was a cluster of cells with CF < 2 kHz, but very few cells below 1 kHz. A high-CF bias (compared to the behavioral audiogram) is also present in the rat (Zhang and Kelly 2006a). This is perhaps less obvious in the rabbit (Batra and Fitzpatrick 1999), although even there very few neurons with CF < 700 Hz were present. Except for the sensitivity to interaural time differences in the onset response of some neurons of the rabbit (Batra and Fitzpatrick 1999, 2002b), there is thus little evidence for the processing of stimulus fine structure in the VNLL, which likely limits its role in temporal pitch processing (Langner 2005; Oertel and Wickesberg 2002). First-spike latencies of sustained and onset neurons to CF

Fig. 13. A: spectrotemporal receptive field (STRF) analysis performed on the responses of a VNLL neuron to frozen white noise. CF of the neuron, obtained from its responses to single tones, is indicated by the arrow (14.8 kHz). The scale of the color code is arbitrary. B: CF estimated from peak values of STRFs (CFNoise) plotted against CF estimated from responses of the same neurons to single tones (CFTones).

and 0.262, respectively. Minimum responses. Only Fourier amplitude is normalized to the maximum SC value of the SAM indicated by increasing thickness of the dashed lines and circles (e.g., of the same neurons to SAM sounds at 3 levels (30, 50, and 70 dB SPL), A–J)

transform amplitude of the reverse-correlation waveforms shown in Fig. 14, (1991) (Fig. 4, A–J). The continuous lines in Fig. 15. Continuous lines in A–J represent the square root of the Fourier transform amplitude of the reverse-correlation waveforms shown in Fig. 14, A–J. Dashed lines with circles indicate the rMTFs obtained from the response of the same neurons to SAM sounds at 3 levels (30, 50, and 70 dB SPL), indicated by increasing thickness of the dashed lines and circles (e.g., H). The Fourier amplitude is normalized to the maximum SC value of the SAM responses. Only r values with 5% significance levels are displayed (t-test). Minimum P values computed from the data in E–H equal 0.0576, 0.233, 0.122, and 0.262, respectively.

tones did not show a clear dependence on CF (Fig. 3A) and were remarkably precise for many onset neurons (Fig. 3B). A small proportion of onset neurons could be classified as "constant latency," as defined by Covey and Casseday (1991) (Fig. 4, A and B). Six additional neurons (3 onset and 3 sustained) were considered as near constant-latency neurons, since changes in first-spike latency were <1 ms but for stimulus level 20–40 dB re threshold.

Frequency tuning is narrower in ANFs than in VNLL neurons. Analysis performed on frequency tuning data in Fig. 2J reveals that Q_{10} values of ANFs are larger than those computed from VNLL neurons, at the 1% level of significance. Similarly, Batra and Fitzpatrick (2002b) concluded that VNLL tuning in rabbits is broader than in the auditory nerve.

Batra and Fitzpatrick (1999) discussed at length the mechanisms that might shape the VNLL discharge patterns to tones. Excitatory input to the VNLL arises mostly from stellate, octopus, and bushy cells in the contralateral VCN (reviewed by Oertel and Wickersberg 2002). Whereas stellate and bushy cells project to all parts of the VNLL, octopus cells project to its ventral part with large terminals (Adams 1997; Schofield and Cant 1997; Smith et al. 2005). Indeed, several response properties of the VNLL are similar to those of onset neurons in the VCN, which can show wide and sometimes multipeaked frequency tuning, well-timed onset responses, and even band-pass tuned rMTFs (Godfrey et al. 1975; Rhode 1994; Rhode and Smith 1986; Smith et al. 2005; Winter and Palmer 1995). Unfortunately, the designation “onset response” is used in two ways, which often obscures classification: as “rate” onset, i.e., a restriction of spiking to the onset of the stimulus (the definition used here), or as “time” onset, i.e., highly reproducible timing of the first spike after stimulus onset. The two properties often occur simultaneously, which probably has led to the terminological confusion. For onset neurons in the VCN, the correspondence between anatomy and physiology and the equivalence across species has not been fully worked out, but it is clear that octopus cells provide a strong excitatory input to the VNLL and that they show many commonalities with onset neurons in the VNLL. Thus the latter likely inherit some of their properties from these inputs.

Of course, the striking temporal properties of VNLL neurons do not preclude the existence of interesting spectral properties, but to our knowledge these have not been explored. Portfors and Wenstrup (2001) found sensitivity to combination tones in neurons with W-shaped tuning curves in the INLL of the mustached bat. The W-shaped pattern in the tuning curve of some VNLL neurons might indicate the capability of the neurons to perform across-channel comparisons, perhaps akin to the sensitivity demonstrated in onset neurons of the VCN in the guinea pig (Winter and Palmer 1995).

Binaural properties of VNLL neurons. The proportion of VNLL neurons considered binaural was relatively small in Aitkin et al. (1970) and Covey and Casseday (1991) but was larger (50%) in our sample, as in Guinan et al. (1972a, 1972b) and in other recent studies (Batra and Fitzpatrick 1999, 2002a; Zhang and Kelly 2006a). Binaural interaction was shown to be weak, however, particularly compared with the LSO (Fig. 16), although not negligible for real-world ILDs. Stronger binaural VNLL regions might have been missed because of our approach in finding the VNLL (see METHODS). Sources of inhibition are discussed by Zhang and Kelly (2006a) and include projections from the superior olivary complex as well as intrinsic circuitry. Also, the weak binaural interaction in the VNLL is perhaps inherited from the VCN (Joris and Smith 1998; Recio-Spinoso 2005; Shore et al. 2003). Interestingly, the sign of the binaural interaction is dominantly EI (contralateral ear excitatory, ipsilateral ear inhibitory), similar to high-CF neurons in the IC. Since the VNLL projection to the IC is ipsilateral and inhibitory, this commonality in binaural sign raises the question as to the exact contribution of the VNLL’s binaurality to that of the IC.
**Possible functional role.** The prominence of temporally tight onset responses and the coding of modulations in rate and synchronization tie in, at a qualitative level, with recent ideas on coding. Onset responses may be important as epoch time markers, i.e., as “reference” signals for chunks of sound; sustained responses may be important for the coding of envelope shape. This distinction is along the lines suggested for the IC (Zheng and Escabi 2008). A role for sustained neurons in the coding of envelope shape is congruent with the consistency in envelope coding across very different stimuli in these neurons (Fig. 9, Fig. 15).

First-spike latency has been proposed as a code in the auditory midbrain and cortex for various acoustic features (Chase and Young 2007; Furukawa et al. 2000; Stecker and Middlebrooks 2003; Zohar et al. 2011). A general issue with such codes is that spike timing may be striking to the experimenter—who can reference to the stimulus—but is useful to the brain only if it also has a neural reference signal. In binaural hearing, the reference signals are the spike trains evoked at the other ear. For monaural hearing, the invariance of latency of VNLL onset neurons with CF and SPL, relative to other neurons, makes these neurons suitable as population markers for stimulus onset, as suggested by Chase and Young (2007).

A first-spike latency code is simplest to envisage for stimuli gated in silence, but the concept is generic and may be applicable to more common situations, such as sustained communication signals in the presence of other signals or background noise. Such natural sounds invariably contain modulations in amplitude and frequency, and additional modulations arise by the summation of different sources and by cochlear filtering. These occur on different timescales and provide important cues for scene analysis (Sinex 2005; Sinex et al. 2002, 2005). The VNLL sets up spike trains that lock on to these modulations at different timescales, as expressed in their MTF.

The advantage of having such reference signals in the form of inhibitory rather than excitatory spike trains is unclear, but it is of note that leading inhibition is a response feature that has been found at various levels including the midbrain and even the VNLL itself (Carney and Yin 1989; Covey et al. 1996; Kuwada et al. 1986, 1997; Nayagam et al. 2005; Smith et al. 1993; Steinberg and Pena 2011) and that it has been proposed to contribute to feature binding (Nayagam et al. 2006).

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: A.R.-S. and P.X.J. conception and design of research; A.R.-S. and P.X.J. performed experiments; A.R.-S. analyzed data; A.R.-S. and
P.X.J. interpreted results of experiments; A.R.-S. and P.X.J. prepared figures; A.R.-S. drafted manuscript; A.R.-S. and P.X.J. edited and revised manuscript; A.R.-S. and P.X.J. approved final version of manuscript.

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