Responses of Neurons in the Ventral Nucleus of the Lateral Lemniscus to Sinusoidally Amplitude Modulated Tones

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Batra, Ranjan. Responses of neurons in the ventral nucleus of the lateral lemniscus to sinusoidally amplitude modulated tones. J Neurophysiol 96: 2388–2398, 2006. First published August 9, 2006; doi:10.1152/jn.00442.2006. Fluctuations in the amplitude of a sound play an important role in our perception of pitch and acoustic space, but their neural analysis has not been fully elucidated. The ventral nucleus of the lateral lemniscus (VNLL) has been implicated in the processing of such temporal features of a sound. This study examines responses of neurons in the VNLL of unanesthetized rabbits to sinusoidally amplitude modulated tones, a type of stimulus that has often been used to investigate encoding of temporal information. Modulation transfer functions of responses were calculated in two ways: based on discharge rates (rMTFs) and on synchronization to the envelope (tMTFs). Among the variety of rMTFs, two types were readily identifiable: flat and band-pass. The responses of neurons exhibiting these types of rMTF differed in several ways. Neurons with flat rMTFs typically had moderate rates of spontaneous activity, sustained responses to short tone bursts, and low-pass or band-pass tMTFs. Neurons with band-pass rMTFs typically had low spontaneous activity, onset responses to short tone bursts, and flat tMTFs. The vast majority synchronized strongly to the modulation envelope. The best modulation frequencies of neurons with band-pass rMTFs extended from 14 to 283 Hz. The presence of neurons with band-pass rMTFs in the VNLL suggests that this nucleus plays a role in converting the temporal code for modulation frequency used in lower structures into a rate-based code for use higher in the auditory pathway. The substantial number of neurons with more complex modulation transfer functions indicates that the VNLL has other functions.

INTRODUCTION

The auditory system is adept at processing the temporal features of sounds. Temporal information resides in two aspects of a sound: the rapid cycle-by-cycle pressure fluctuations and the slower, superimposed modulation of the amplitude (AM) of the sound. We perceive these slower fluctuations as a roughness or even as a pitch, depending on the modulation rate (Joris et al. 2004). Neural analysis of AM likely contributes to our ability to interpret speech and to perceive the pitch of complex sounds.

Physiological and psychophysical evidence suggest that sensitivity to AM is mediated by a bank of neural filters in which each filter is sensitive to a different rate of modulation (reviewed by Joris et al. 2004). A key question is where such filters might be located in the auditory system. The cochlear nucleus contains neurons that discharge in synchrony to sinusoidally amplitude modulated (SAM) tones, and some of these exhibit maximal synchrony at one or another modulation frequency (reviewed by Frisina 2001; Joris et al. 2004; Rhode and Greenberg 1992). Most neurons exhibit only a variation in the degree of synchrony to the SAM tone and not a variation in the discharge rate. Further up the pathway, in the inferior colliculus, synchrony to higher modulation frequencies is absent. Instead, there are neurons that discharge maximally to a preferred modulation frequency (reviewed by Frisina 2001; Joris et al. 2004). This change in the encoding of AM between the cochlear nucleus and the inferior colliculus, coupled with the direct projection from the former to the latter, led to the suggestion that the putative bank of filters is located in the cochlear nucleus but based on a temporal code, and that this temporal code is translated into a rate code in the inferior colliculus (Hewitt and Meddis 1994). Alternatively, it was suggested that the putative filters are constructed in the inferior colliculus (Schreiner and Langner 1988).

There are, however, indirect pathways through which information is transmitted from the cochlear nucleus to the inferior colliculus and a bank of filters sensitive to different modulation frequencies may reside in one of these. Of particular interest is the pathway through the ventral nucleus of the lateral lemniscus (VNLL). This nucleus receives its main input from the contralateral ventral cochlear nucleus (earlier work summarized in Glendenning et al. 1981; for a review of more recent material see Oertel and Wickesberg 2002). The ventral cochlear nucleus is known to transmit temporal information, specifically, the cues necessary for sound localization. It may transmit such information to the VNLL as well. The VNLL in turn sends a large projection to the inferior colliculus.

The present study examines responses of neurons in the VNLL to SAM tones, focusing on their filtering abilities across modulation frequency. The main findings are that in the VNLL there are neurons that discharge maximally to a preferred modulation frequency and that the preferred modulation frequencies of different neurons span a wide range encompassing several octaves.

METHODS

The experiments described herein were conducted at two locations: the University of Connecticut Health Center and the University of Mississippi Medical Center. The equipment and techniques differed somewhat at the two locations, so both are described below. Data...
collected at the two locations did not differ in any obvious way and were therefore pooled.

Ten adult Dutch-belted rabbits (about 2 kg) were used to record responses in the VNLL: six in Connecticut and four in Mississippi. In addition, latencies for neurons in the inferior colliculus are reported from one other rabbit in Mississippi. All surgery and procedures performed on the rabbits were approved by the Animal Care Committee in Connecticut and by the Institutional Animal Care and Use Committee in Mississippi. All animals had clean ears. Three previous publications (Batra and Fitzpatrick 1997, 1999, 2002) were based on data from the six animals used in Connecticut, but these publications did not report the responses to SAM tones.

**Surgery**

Animals were prepared for recordings as previously described (Batra and Fitzpatrick 1999). Two surgeries were performed: one to implant the restraint rod and the other for the craniotomy. Aseptic techniques were used for both procedures. Anesthesia was induced using a mixture of ketamine and xylazine (35 and 5 mg/kg, administered intramuscularly [im] and maintained with supplemental doses delivered intravenously or im as often as required. In the first surgery, the skin and fascia overlying the dorsum of the cranium were retracted and a short length of square, brass stock was attached to the cranium to the left of midline using fine screws and dental acrylic. The brass stock served as the restraint bar and was clamped during recording sessions. The cranium overlying the right VNLL remained exposed. Either before or after the surgery, custom earmolds were made. Each finished ear mold had a tube extending through it for delivery of sound. After implantation of the restraint rod the rabbit spent about 1–5 wk acclimating to the restraint procedure and ear molds. After the acclimation period the second surgery was performed. A craniotomy (about 4 mm²) was made over the right VNLL. Antibiotic was applied to the exposed dura and the craniotomy sealed with sterilized elastopolymer (Sammons, Preston, and Rolyan, Bolingbrook, IL).

**Recording procedure**

After several days for recovery, recordings were initiated. The rabbit sat in a couch in a double-walled soundproof booth. It was restrained by ensheathing it in a body stocking and by nylon straps. The restraint rod was clamped. Lidocaine (2%) was used to desensitize the dura. The dura was then pierced by a hypodermic needle and the electrode was advanced into the brain through the opening. The dura was then pierced by a hypodermic needle and filtered. Action potentials of single neurons were distinguished if the rabbit fidgeted. Each rabbit took part in 20–30 recording sessions. The electrical activity encountered by the electrode was amplified and filtered. Action potentials of single neurons were distinguished based on uniformity of size and waveform, discriminated using a time-amplitude window discriminator (BAK Electronics, Germantown, MD) and timed with a resolution of 10 µs (Connecticut) or 1 µs (Mississippi). Spontaneous activity was recorded during a 10-s silent period for most neurons. In a few neurons, it was estimated from the silent interval between stimulus repetitions when the stimulus was of low intensity.

**Acoustic stimulation and calibration**

Acoustic stimuli were produced digitally by dual synthesizers, one for each ear. In Connecticut, the synthesizers used were those designed at the University of Wisconsin (Rhode 1976) and were controlled by a PDP-11 computer running custom software that also controlled data collection. The digitized signals were passed through attenuators, amplified, and transmitted to 8-Ω DT-48 earphones (Beyerdynamic, Hicksville, NY). In Mississippi, stimuli were synthesized by a Tucker Davis Technologies (TDT) System 2 (Alachua, FL) controlled by software written by Dr. Marcel van der Heijden (Utrecht, The Netherlands). The stimuli were digitized at 20, 50, or 125 kHz, whichever was >2.5 × the highest frequency in the stimulus. The analog signals were passed through a filter with a cutoff of 10, 26, or 65 kHz (TDT FT-6 filters), whichever was closest to the Nyquist frequency of the digitization rate. Switching was accomplished by a TDT SS-1 unit. The signals were then attenuated (TDT PA4) so that they were at the desired intensity level, amplified (TDT HB6 headphone buffer), and transmitted either directly to 8-Ω DT-48 earphones or to 200-Ω DT-48 earphones by a passive network designed to flatten the low-frequency part of the response of the earphones. The earphones were connected to the sound delivery tubes that ran through the ear molds, producing a sealed system.

The procedure for acoustic calibrations evolved over the course of the experiments. In Connecticut, the intensity levels of tones (about 50 Hz to 50 kHz) were measured in dB SPL (re 20 µPa), either deep in the meatus or opposite the tympanum (details in Batra and Fitzpatrick 1999). In Mississippi, calibrations were performed in the meatus about 2 cm from the tympanum by recording presentations of noise bursts with progressively higher frequency content. Frequencies from about 50 Hz to 21 kHz were calibrated in this way. From 21 to 50 kHz, a standard calibration was used that was obtained by attaching a calibrated microphone to the end of the sound delivery tube by a short stretch of plastic tubing. Neurons with best frequencies (see following text) of 19–23 kHz were not studied in Mississippi.

Best frequencies were determined using short tone bursts (75 ms, repeated every 200 ms) of constant intensity. The SAM tones were presented to the contralateral ear and were typically 1,100 or 5,100 ms in duration, repeated every 1,300 or 1,700 ms, or 5,300 or 5,700 ms, respectively. The longer duration was used for modulation frequencies 25 Hz. The carrier frequency was set to the best frequency of the neuron. All stimuli had linear rising and falling edges of 4-ms duration. The stimulus system in Connecticut used the calibration to adjust the level and carrier phase of pure tones and SAM tones. In Mississippi, the system adjusted the amplitudes and phases of all components of the SAM tone, ensuring that a sinusoidal envelope was present in the vicinity of the tympanum. For each neuron studied, an initial sequence was recorded using the shorter stimulus, a modulation depth of 100%, a typical intensity of 50–70 dB SPL, and modulation frequencies that usually ranged from 6 or 25 to 800 or 1,600 Hz, varied in logarithmic steps. If the recording persisted and if responses to lower frequencies had not already been recorded, a second sequence was taken using the longer stimulus and frequencies from 3.1 to 25 Hz. Responses to 25 Hz, measured using stimuli of both durations, served as a check on the system, ensuring that a sinusoidal envelope was present in the vicinity of the tympanum. For each neuron studied, an initial sequence was recorded using the shorter stimulus, a modulation depth of 100%, a typical intensity of 50–70 dB SPL, and modulation frequencies that usually ranged from 6 or 25 to 800 or 1,600 Hz, varied in logarithmic steps. If the recording persisted and if responses to lower frequencies had not already been recorded, a second sequence was taken using the longer stimulus and frequencies from 3.1 to 25 Hz. Responses to 25 Hz, measured using stimuli of both durations, served as a check on whether differences were present. Typically, differences were small and the responses were averaged together. Additional frequencies were sometimes tested as well. Sequences were initially run in descending order. In some cases, sequences were also run in ascending or random order to check for adaptation.

**Localization of recording sites**

The locations of most neurons were reconstructed based on a few lesions or dye injections made at the end of recordings in each animal (for a detailed description of the procedures for histology and reconstruction see Batra and Fitzpatrick 1999). For the remaining neurons, locations were marked directly with an injection of dextran tagged with one of a variety of fluorescent labels or with biotin, or with an injection of fluorogold.
Classification of modulation transfer functions

Modulation transfer functions (MTFs) of neurons were calculated from the responses to SAM tones over a range of modulation frequencies. This range always covered 25–400 Hz or half the carrier frequency, whichever was lower. Any responses to modulation frequencies > half the carrier frequency were excluded from analysis.

Two types of MTFs were calculated: one based on the driven discharge rate (rMTF; i.e., the average discharge rate above the level of spontaneous activity) and the other based on the synchronization coefficient (SC) or, equivalently, the vector strength (Goldberg and Brown 1969; as modified in Kuwada et al. 1987) (tMTF). In all calculations of discharge rates, SCs, and cycle histograms, the initial 100 ms of the response was discarded to avoid any effects of transients.

The rMTFs were classified using a scheme that was based on the different types of possible filter characteristics. This scheme was similar to that used by Langner and Schreiner (1988). The classification process consisted of three steps. In the first step, the MTFs were classified into one of six categories: flat, band-pass, band-reject, low-pass, high-pass, and negative driven rate. A rMTF was considered flat if the driven rate was never <50% of the maximum rate across frequency. If the rate declined below this criterion both below and above the frequency at which it was a maximum, the rMTF was initially classified as band-pass. The band-reject, low-pass, and high-pass categories were defined similarly, also based on a 50% criterion.

Neurons in the negative driven rate category responded with suppression of the spontaneous activity at all modulation frequencies.

The second step was intended to weed out MTFs that had complex shapes or appeared based on erratic responses. In this step, neurons were removed from the band-pass, high-pass, and low-pass categories if they had responses at fewer than three frequencies in the vicinity of the maximum that were above the criterion, and these elevated responses could not be verified from other sequences taken during the recording. Neurons with band-reject MTFs were removed if they had fewer than three responses below the criterion in the vicinity of the minimum, which could not be otherwise verified. Neurons with MTFs that had secondary peaks or troughs based on the 50% criterion were removed as well.

The MTFs of the excluded neurons were divided into two groups based on their maximum driven rate: those with driven rates >10 impulses/s were classified as “Indeterminate” and the remainder as “Weak and Irregular.” The latter category also received neurons that had no sustained responses to SAM tones. In the third and final step, each MTF was viewed to determine whether its shape conformed to its designated category. If not, it was moved to a more appropriate category. In practice, the classification of only a few neurons was changed in the final step (roughly 6%). All of these neurons were transferred either into, or out of, the Indeterminate or Weak Responses category.

The tMTFs were initially calculated from responses that were significantly synchronized to the modulation frequency (Rayleigh test of uniformity, \( P < 0.001 \); Mardia and Jupp 1999). However, these tMTFs were difficult to classify because of the concurrent variations in synchrony and discharge rate with modulation frequency.

To understand the problem, consider the two tMTFs in the top panels of Fig. 1. The tMTFs based on significantly synchronized responses (Fig. 1, top panels, unfilled circles) are both flat in that neither falls below the criterion level (arrows) equal to 50% of the highest SC. However, the underlying responses of the two neurons at high modulation frequencies were different. The absence of significant synchrony at these modulation frequencies in the neuron of Fig. 1A is because its discharge rate declined to near zero (Fig. 1A, bottom). In contrast, the neuron of Fig. 1B continued to discharge at a moderate rate at these modulation frequencies (Fig. 1B, bottom), although the SC declined to a value at which it was no longer statistically significant. Although the tMTF of the latter neuron was nominally classified as being flat, its SC could have declined below the 50% criterion level used to distinguish flat from low-pass, and the tMTF might be better classified as low-pass.

To determine the extent of the decline in SC at higher modulation frequencies for these two neurons, the SC that would be barely statistically significant (Rayleigh test of uniformity, \( P < 0.001 \)) was calculated at each frequency (Fig. 1, top panels, filled circles). This SC was effectively a background noise level and was calculated by interpolation from a table of critical values for the statistical test. The recorded SC lay below the noise level when it was not statistically significant. When this was the case, the recorded SC was an inadequate measure of the true SC because, at the chosen confidence level, it could have occurred randomly in the absence of any synchrony whatever to the modulation envelope. However, the true SC must also have lain below the background noise level or else it would have been detected as being statistically significant. Thus if the noise level remained below the 50% criterion (Fig. 1B, top), then the true SC must have been below the criterion as well. On the other hand, if the noise level rose above the 50% criterion (Fig. 1A, top), then the value of the true SC relative to this criterion would remain unknown. For this reason, the tMTF based on statistically significant SCs was extended by joining it with the noise level at frequencies at which the response was not significantly synchronized to the modulation (Fig. 1, dotted lines). These extended tMTFs were then classified using a process similar to that for MTFs. If the extended tMTFs declined at higher modulation frequencies, they were classified as either low-pass or band-pass, depending on their behavior at low frequencies. Conversely, if they did not decline they were classified as flat or high-pass. The extended tMTFs were used only for categorization and for calculation of the high-frequency cutoff (Table 1).

FIG. 1. Construction of modulation transfer functions (MTFs) based on the synchronization coefficient (tMTFs). A and B, top: tMTFs of 2 neurons. Bottom: corresponding MTFs based on rate (rMTFs). tMTFs were calculated from responses to sinusoidally amplitude modulated (SAM) tones at different modulation frequencies and the same carrier frequency. They were initially constructed by joining the synchronization coefficients (SCs) of responses that were statistically synchronized to the modulation (Rayleigh test of uniformity, \( P < 0.001 \)) (unfilled circles, solid lines). tMTFs were then extended by joining them (dotted line) to the effective background noise level (filled circles) at frequencies to which the response was not significantly synchronized (see text for calculation of background noise level). Such extended tMTFs were used for classification and for calculation of cutoff frequencies (Table 1). Arrow: criterion equal to half the maximum SC in the tMTF. A: neuron with a tMTF that does not fall below the criterion, and is classified as flat. B: neuron with a tMTF that falls below the criterion at higher frequencies and is classified as low-pass.
A difference between the classification schemes of rMTFs and tMTFs was that the "Negative Driven Rate" category, which does not apply to tMTFs, was replaced by a category for neurons that synchronized significantly to at least three modulation frequencies (Synchrony Absent).

RESULTS

These results are based on the responses of 132 neurons that were tested with SAM tones. The best frequencies of the neurons ranged from 700 Hz to 45 kHz with the central two thirds lying between 2.7 and 7.1 kHz. Of the neurons tested, 21 responded weakly and irregularly to SAM tones (see Classification of modulation transfer functions in METHODS). The majority of such neurons (15/21) responded only at the onset of a SAM tone. Neurons that responded weakly and irregularly are not considered further.

All neurons studied were in one or another division of the VNLL. In the rabbit, the VNLL has three main divisions: 1) a lateral, cell-dense division; 2) a medial division, consisting of scattered neurons intercalated in the medial limb of the lemniscus; and 3) a dorsal division that is sparser than the lateral division (Batra and Fitzpatrick 1997, 1999). The neurons of the medial division extend into the anterior limb of the lemniscus. The dorsal division probably corresponds to the intermediate nucleus of the lateral lemniscus that has been defined in other species (Glendenning et al. 1981; Schofield and Cant 1997). The present terminology for this region follows that of Adams (1979).

Among the variety of response patterns to SAM tones across modulation frequencies displayed by different neurons in the VNLL, two were notable (Fig. 2). Neurons displaying the first pattern synchronized well to SAM tones at lower modulation frequencies (Fig. 2A, top). At progressively higher modulation frequencies, the synchrony declined, but the steady discharge was maintained (Fig. 2A, second to fourth panels). At the highest frequency, the steady discharge remained, but it was not synchronized to the modulation (Fig. 2A, fifth panel and cycle histogram in inset).

Neurons displaying the second pattern synchronized more strongly to SAM tones at lower modulation frequencies (Fig. 2B, first to third panels) than those displaying the first pattern. At higher modulation frequencies, there was a steady discharge (Fig. 2B, fourth and fifth panels). The discharge rate declined at these higher frequencies, but the action potentials were still strongly synchronized to the modulation (Fig. 2B, fifth panel, cycle histogram in inset).

MTFs based on rate

The differences between the two patterns of response illustrated in Fig. 2 were reflected in the corresponding rMTFs (Fig. 3, A and B). The neuron of Fig. 2A had a flat rMTF (Fig. 3A, circles), whereas the neuron of Fig. 2B had a band-pass rMTF (Fig. 3B, circles). Neurons with flat and band-pass rMTFs had discharge rates that varied over a wide range. The best modulation frequencies of neurons with band-pass rMTFs varied over a wide range as well.

<table>
<thead>
<tr>
<th>Category of Neuron</th>
<th>Flat rMTF</th>
<th>Bandpass rMTF</th>
<th>Full Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-dB Upper cutoff, rMTF, Hz</td>
<td>—</td>
<td>234 ± 236 (23)</td>
<td>292 ± 346 (42)</td>
</tr>
<tr>
<td>Peak frequency, rMTF, Hz</td>
<td>96 ± 86 (24)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6-dB Upper cutoff, tMTF, Hz</td>
<td>397 ± 220 (26)</td>
<td>517 ± 257 (8)</td>
<td>422 ± 246 (59)</td>
</tr>
<tr>
<td>Highest significantly synchronized frequency, Hz; P &lt; 0.001</td>
<td>580 ± 322 (27)</td>
<td>367 ± 334 (24)</td>
<td>456 ± 328 (102)</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = number of neurons (in parentheses). The cutoff frequencies were not calculated for neurons with MTFs that either did not decline or had highly irregular declines at high frequencies. In particular, the 6-dB upper cutoff of the tMTF could not be calculated for the majority of neurons with bandpass rMTFs because they had flat tMTFs.

A difference between the classification schemes of rMTFs and tMTFs was that the “Negative Driven Rate” category, which does not apply to tMTFs, was replaced by a category for neurons that synchronized significantly to at least three modulation frequencies (Synchrony Absent).

FIG. 2. Responses of 2 archetypal neurons in the ventral nucleus of the lateral lemniscus (VNLL) to SAM tones. A and B: 2 neurons. Responses are shown in the form of peristimulus time histograms (PSTHs). Lines under top panels indicate stimulus duration. Modulation frequencies are noted at top right in each panel. Insets, bottom panels: corresponding cycle histograms based on the modulation period. A: 4-kHz carrier, 60 dB SPL. B: 4-kHz carrier, 70 dB SPL. Repetition interval: 1.3 s.
Other neurons in the VNLL had rMTFs with other shapes, such as low-pass and high-pass (Fig. 3, C and D). Many neurons had rMTFs that were multipeaked or had other complicated shapes that were difficult to define. These rMTFs were classified as indeterminate (Fig. 3 E) (see METHODS for details of classification).

Flat and band-pass were the two most common of the readily definable types of rMTFs (Fig. 3 F, Flat and BP, respectively), but there were many neurons with indeterminate rMTFs as well. Even together, the flat and band-pass rMTFs were a minority in the sample of neurons studied (39% or 52/132 neurons).

Some of the classes of rMTFs were associated with particular discharge patterns to short tone bursts (Fig. 4). The types of discharge patterns encountered in the VNLL of unanesthetized rabbits were described in an earlier report (Batra and Fitzpatrick 1999), and several are illustrated along the abscissa of Fig. 4 in the form of peristimulus time histograms (PSTHs). From the left, these are the onset, short-latency sustained (SL-sustained), transient, long latency, and inhibited.

Most neurons with band-pass rMTFs had onset PSTHs (Fig. 4 A), area of circles is proportional to number of neurons), but several had SL-sustained PSTHs. All neurons with flat rMTFs had SL-sustained PSTHs. Neurons with negative driven rates in response to SAM tones had either onset or inhibited PSTHs. Indeterminate rMTFs were concentrated among neurons with SL-sustained and long-latency PSTHs.

Few neurons with band-pass rMTFs are found lower in the auditory pathway. For this reason, their properties were explored in more detail. The peak modulation frequencies of these neurons spanned a range of over four octaves (Fig. 5A).

The preponderance of onset responses to short tones in neurons with band-pass rMTFs suggests that this pattern of
response to SAM tones arises because each cycle of modulation acts like a tone burst and triggers an onset response. If this is the case, then the discharge rate to SAM tones should increase linearly with modulation frequency below the peak, as an increasing number of cycles in a fixed interval of time trigger more onset responses. A linear increase is indicated by a slope of 1 in a double-logarithmic plot (Fig. 5B, dashed line). Shallower and steeper curves indicate, respectively, decelerating and accelerating changes in discharge rate with modulation frequency.

Some neurons with band-pass rMTFs had linear low-frequency slopes (Fig. 5B, dotted lines). However, others showed a decelerating pattern (Fig. 5B, solid lines) and still others an accelerating pattern (dot–dash lines). Across neurons, the slopes of the double-logarithmic plots were centered at 1, but some neurons had high-pass or band-reject tMTFs (Fig. 6, B and E). In some neurons, the part of the tMTF below the cutoff frequency was quite flat (Fig. 6, dot–dash line), but more frequently it had a gradually rising slope (other lines). Flat tMTFs tended to be similar to one another, with high SCs and a gradual decline in synchrony at higher modulation frequencies (Fig. 6B). Band-pass tMTFs were similar to those that were low-pass, except that the low-frequency slope was somewhat steeper (Fig. 6C). The peaks of band-pass tMTFs were concentrated between 100 and 200 Hz: two thirds of them lay in this range. Indeterminate tMTFs had a variety of irregular shapes (Fig. 6D).

Neurons with low-pass tMTFs were most common (Fig. 6E, LP), followed by those with tMTFs that were flat or indeterminate. In contrast to the distribution of rMTFs, almost no neurons had high-pass or band-reject tMTFs (Fig. 6E, HP and BR, respectively).

Neurons with band-pass and low-pass tMTFs may form one functional category. The upward slope of the low-frequency limb has been ascribed to adaptation or to on-frequency inhibition. Variation in the strength of these processes could account for the variation in slope. The tMTFs of many neurons in the cochlear nucleus tend to change from low-pass to band-pass as the intensity is raised (Frisina et al. 1985, 1990; Kim et al. 1990; Møller 1972; Rhode and Greenberg 1992). To determine whether this might also happen in the VNLL, 13 neurons with either band-pass or low-pass tMTFs at higher intensities were tested at lower intensities as well (Fig. 7). The changes with sound level spanned a broad spectrum. Some neurons maintained the low-pass or band-pass characteristic of their tMTFs as the sound level was varied (Fig. 7, A and B, respectively); others had tMTFs that changed from low-pass to band-pass as the sound level was raised (Fig. 7C); still others had tMTFs that underwent the opposite change (Fig. 7D). Overall, nearly half of the neurons (6/13) had tMTFs that switched between band-pass and low-pass, supporting the idea that these two groups in the present classification scheme are really one functional category.

The rMTF and tMTF of the neuron with the responses illustrated in Fig. 2A suggest that neurons that maintain a constant firing rate across modulation frequency (i.e., those with flat rMTFs) tend to have low-pass tMTFs. The rMTF and tMTF of the neuron with the response illustrated in Fig. 2B suggest that neurons that are tuned by rate to a particular modulation frequency (i.e., those with band-pass rMTFs) tend to have flat tMTFs. This was essentially the case (Fig. 8). Most neurons with flat rMTFs had either band-pass or low-pass tMTFs (Fig. 8, second row, 20/28 flat rMTF neurons), whereas most neurons with band-pass rMTFs had flat tMTFs (Fig. 8, first row, 14/24 band-pass rMTF neurons).

The only other class of rMTF that appeared to be associated with one or more classes of tMTF were neurons with negative driven rates (Fig. 8, third row). These had either flat tMTFs or did not synchronize to the modulation. Neurons with indeterminate rMTFs were distributed broadly across the different classes of tMTFs, with a slight concentration in indeterminate tMTFs.
Other differences between neurons with flat and band-pass rMTFs

The responses of neurons with band-pass and flat rMTFs differed from one another in several respects besides the difference in the shape of the rMTF and the pattern of response evoked by short tone bursts (Fig. 9). To begin with, the two types of neurons differed in their spontaneous activity and their maximum discharge rate (Fig. 9, A and B). Most neurons with band-pass rMTFs had extremely low spontaneous rates (<10/s), whereas those with flat rMTFs tended to have higher spontaneous activity. The difference in driven discharge paralleled the difference in spontaneous activity.

Neurons with band-pass rMTFs synchronized more strongly to SAM tones (Fig. 9C). For over two thirds of these neurons (71%, 17/24), the largest SCs were >0.9, whereas no neurons with flat rMTFs had such high SCs. However, neurons with flat rMTFs could typically follow modulations at higher rates (Fig. 9D). Note that this comparison is based on the highest modulation frequency at which significant synchrony (P < 0.001, Rayleigh test of uniformity; Mardia and Jupp 1999) was observed. Solid lines: overall distributions across neurons in the sample. Dotted line in D: neurons above this line synchronized to the modulation at the highest frequency tested. E: difference in best frequencies. Maximum driven responses of each neuron are the maximum values of their respective rMTFs. Total neurons and neurons with band-pass and flat rMTFs—A: 111, 24, 28; B: 102, 24, 28; C: 102, 24, 27; D: 102, 24, 27; E: n/a, 24, 28. In B, neurons in the negative driven rate class have been omitted. In C and D, neurons in the Synchrony Absent class have been excluded.

rMTFs could typically follow modulations at higher rates (Fig. 9E). Note that this comparison is based on the highest modulation frequency to which significant synchrony was observed (Rayleigh test of uniformity, P < 0.001; Mardia and Jupp 1999). More conventional measures, such as the cutoff frequency, cannot be used because the SC of many neurons with band-pass rMTFs remains high even at the highest frequency to which a response is present.

Neurons with band-pass and flat rMTFs also differed in their best frequencies (Fig. 9E). Neurons with band-pass rMTFs tended to have lower best frequencies than those with flat rMTFs. Differences between the band-pass and flat neurons in spontaneous and driven activity, the largest SC displayed, the highest frequency to which synchrony occurred, and in best frequencies were statistically significant (Kolmogorov–Smirnov test, one- and two-tailed, P < 0.01; Siegel 1956).
The various differences between neurons with band-pass and flat rMTFs did not appear to be a consequence of a dependency of the relevant parameter on best frequency coupled with the difference in average best frequencies between the two groups. This possibility was examined by calculating the linear regression of various features against the best frequency for the two types of neurons separately, and testing for slopes that differed significantly from zero \( (P < 0.05, t\text{-test}; \text{DeGroot 1975}) \). The reasoning was that if, say, the difference in spontaneous rate between the two types of neurons was a consequence of the difference in best frequency, then there should be an overall dependency of spontaneous rate on best frequency that would be apparent for both types of neurons. A dependency was found for the highest modulation frequency at which neurons with band-pass rMTFs synchronized to the envelope; however, this dependency was absent when only neurons with best frequencies \( > 2 \text{ kHz} \) were considered. Below this frequency, the carrier frequency (which was set to the best frequency) limits the range over which modulation frequencies can be tested. There was also a dependency of the spontaneous rate on best frequency for neurons with band-pass rMTFs, but this dependency was absent for neurons with flat rMTFs. Thus the various differences between the two types of neurons did not appear to be a consequence of the difference in their best frequencies. Using the same approach, it did not appear that the greater synchrony of neurons with band-pass rMTFs was a consequence of their lower spontaneous activity.

The distribution across all the neurons in the sample of the largest SC exhibited by each neuron and the highest modulation frequency to which significant synchrony occurred generally followed the combined distributions of the neurons with band-pass and flat rMTFs (Fig. 9, C and D, solid lines). A few neurons still synchronized to the modulation at the highest frequencies tested (Fig. 9D, above dotted line), so the overall distribution in Fig. 9D is biased slightly to lower frequencies. Statistics of the above measures, and more conventional measures of the highest responsive frequency, are summarized in Table 1. Essentially, synchrony was observed to somewhat higher frequencies than reported in the inferior colliculus, but not as high as observed in the cochlear nucleus (Joris et al. 2004).

The difference in the maximum SC between neurons with band-pass and flat rMTFs implies that they differed in modulation gain as well (Fig. 10). However, the modulation gain depended on the depth of modulation of the SAM tone. For initial measurements of the MTFs, the depth of modulation was 100%. A typical neuron with a band-pass rMTF synchronized to this depth of modulation with a SC that was near 1, at modulation frequencies across its responsive range (Fig. 10A, different symbols). At lower modulation depths, this high level of synchrony was maintained, resulting in a higher gain (Fig. 10C). In neurons with flat rMTFs, the SC usually declined at lower modulation depths (Fig. 10B), but the gain increased nevertheless (Fig. 10, B and D). At a modulation depth of 50\%, a difference in gain between neurons with band-pass and flat rMTFs was still apparent (Fig. 10E), although the gain of each was greater than that at a modulation depth of 100\%.

The phase components of the rMTFs were also examined for any differences between neurons with flat and band-pass rMTFs. The phase delays of the rMTFs of both types of neurons showed a nearly linear increase with modulation frequency. Delays calculated from linear fits showed no obvious difference between the two types of neurons. The delay was \( 7.0 \pm 3.3 \text{ ms} \) (mean \( \pm \text{SD}, n = 103 \)). Similarly, there was no difference in the intercept of the fit with the ordinate. The intercept corresponds to the phase of the modulation that fires an action potential. For both types of neurons, these phases were distributed about the center of the rising edge \( (-0.05 \pm 0.21 \text{ cycles}, \text{vector mean } \pm \text{ circular SD}) \).
The average delay was the same as the previously reported median latency for neurons in the VNLL of the rabbit (Batra and Fitzpatrick 1999). In contrast, the median latency across a small population of neurons in the inferior colliculus was 12.0 ms (n = 27).

**DISCUSSION**

This study examined the responses of neurons in the VNLL to SAM tones. It focused on the MTFs, i.e., the variation in response with the modulation frequency. Based on the MTFs, the VNLL contains two readily identifiable classes of neurons: those tuned by their discharge rate to a particular modulation frequency (band-pass rMTFs) and those with discharge rates that were relatively constant across all modulation frequencies (flat rMTFs). These two classes of neurons differed in several respects. In addition to these two classes, there were many other neurons that had rMTFs with more complex shapes.

**The classification scheme**

The different categories used to classify MTFs were not based directly on the present data, but were rather derived from general engineering principles. Similar categories were used for both rMTFs and tMTFs. The aim was to provide a general overview of the shape of MTFs rather than to define distinct categories. Consequently, it is entirely possible that the categorization of individual neurons could depend on the stimulus parameters used.

Many of the rMTFs of neurons in the VNLL fell into two distinct categories: band-pass and flat. These neurons also differed in their tMTFs. Whereas the tMTFs of the former largely fell into one category (flat), the tMTFs of the latter fell into two: low-pass and band-pass. These two categories of tMTFs likely correspond to one functional class. In the cochlear nucleus, neurons with flat rMTFs are the rule, rather than the exception. There, many neurons have low-pass tMTFs at low intensities that change to band-pass at high intensities (Frisina et al. 1985, 1990; Kim et al. 1990; Møller 1972; Rhode and Greenberg 1992). Similar changes in some neurons of the VNLL were observed in the present study, although considerable variation was present. Such variation may reflect variation between functional types in the cochlear nucleus (Frisina et al. 1990), many of which project to the VNLL.

**Origin of neurons with band-pass rMTFs**

The VNLL represents one of the lowest centers in the auditory system yet studied where a substantial proportion of neurons have band-pass rMTFs, and it also appears to be the lowest center where the peak modulation frequencies of the rMTFs span such a wide range. In the cochlear nucleus, few band-pass rMTFs are seen (Rhode 1994; Rhode and Greenberg 1994). These have been associated with neurons that exhibit the O1- and O2-type responses to short tone bursts. The few O1 neurons that have been studied have a restricted range of peak modulation frequencies between 300 and 500 Hz. The range of peak frequencies for O2 neurons has not yet been reported.

The superior olivary complex may also contain neurons with band-pass rMTFs (Grothe 1994; Kulesza et al. 2003; Kuwada and Batra 1999). These neurons discharge only at the offset of pure tones and are nearly the sole constituent of the superior paraolivary nucleus in the rat (Kulesza et al. 2003). The average rMTF for these neurons has a shallow low-frequency slope and, by the criterion of the present study, individual neurons could be either low-pass or band-pass. Band-pass neurons have also been reported in the medial superior olive of the mustached bat (Grothe 1994), but in most species this nucleus is concerned with binaural comparisons for sound localization.

Neurons with band-pass rMTFs have flat tMTFs, which are rare lower in the auditory pathway. Such tMTFs have been reported only at lower stations for some O1 neurons in the cochlear nucleus (Rhode 1994). They appear to arise from a combination of two factors. First, any high- or low-frequency decline in synchrony that might be present is masked by the decline in the discharge rate. Second, spontaneous rates of these neurons are typically low, preventing measurement of the SC in the absence of an evoked discharge. The maximum SCs of these neurons are high and are similar to, or greater than, those of neurons in lower centers with band-pass rMTFs (Kulesza et al. 2003; Kuwada and Batra 1999; Rhode 1994; Rhode and Greenberg 1994). The high degree of synchrony results in a correspondingly high-amplification gain of the modulation envelope, above that seen in the auditory nerve (Joris and Yin 1992). Such high gain has been observed in some onset neurons of the cochlear nucleus and in the offset neurons of the superior olivary complex (Frisina et al. 1990; Kuwada and Batra 1999).

Given the paucity of neurons with band-pass rMTFs lower in the auditory pathway, it is unlikely that all neurons in the VNLL with this characteristic inherit it from ascending inputs, although some might do so. The octopus cells of the cochlear nucleus, which have been associated with O1 responses to short tone bursts, are known to project to the contralateral VNLL (Adams 1997; Schofield and Cant 1997; Smith et al. 2005). The axons of octopus cells form secure, calyceal synapses in the VNLL. There are also neurons in the VNLL with nonlinear type II membrane properties (Wu 1999; Zhao and Wu 2001), which in other nuclei have been associated with neurons involved in temporal processing (Banks and Smith 1992; Smith 1995; Wu and Oertel 1984). However, the proportion of type II neurons exceeds the proportion of neurons likely supplied by calyceal synapses. Furthermore, the range of modulation frequencies encoded by octopus cells (O2 cells) (Rhode 1994) seems much narrower than that observed in the VNLL. Thus it is possible that many band-pass rMTFs result from processing within the VNLL. It is unlikely that band-pass rMTFs are a consequence of descending influences from the inferior colliculus because of the shorter latency in the VNLL.

Rhode (1994) suggested that band-pass rMTFs arise from the tendency of neurons with this characteristic to discharge only at the onset of a stimulus, which could result in each cycle of modulation being treated as a separate stimulus. The decline in firing rate below the peak frequency is a consequence of the fewer number of modulation cycles in a given interval of time; the decline at higher frequencies reflects the inability of the neuron to follow extremely rapid intensity fluctuations. There are two arguments against this view. First, as Joris (2004) remarked, such a mechanism should not work at low modulation depths. At the very least, the synchrony of the discharge to a particular modulation phase should decline. Such a decline was not evident in the present study. Second, the rising slope
of the rMTF should be linear. For most band-pass neurons of the VNLL, this was not the case. Thus although the onset response may play a role in shaping the rMTF, it is likely that other factors play an equally important part.

There are parallels between the architecture and physiology of the medial superior olive on one hand, and the VNLL on the other. Both receive ladderlike inputs from the spherical bushy cells of the contralateral ventral cochlear nucleus (Beckius et al. 1999) and input from the ipsilateral medial nucleus of the trapezoid body (Casseday et al. 1988; Glendenning et al. 1981; Sommer et al. 1993). Both these sources are well known for carrying temporal information about the sound at the contralateral ear. The medial superior olive contains neurons that synchronize strongly to tones at either ear (Batra et al. 1997; Spitzer and Semple 1995; Yin and Chan 1990). The VNLL contains neurons that synchronize strongly to envelopes at the contralateral ear. In the medial superior olive, there are neurons tuned by rate to a particular interaural delay. In the VNLL there are neurons tuned to a particular modulation frequency. These parallels lead to the notion that the medial superior olive and the VNLL process timing cues by similar mechanisms, but to different ends: the medial superior olive to generate a rate code for interaural delays and the VNLL to generate a rate code for modulation frequency. Such a role for the VNLL is also indicated by its intrinsic architecture and the presence of extensive intrinsic collaterals (Malmierca et al. 1998; Whitley and Henkel 1984).

Influence of band-pass rMTFs in the VNLL on responses in the inferior colliculus

The VNLL provides a major input to the next stage of the auditory pathway, the inferior colliculus. It can therefore influence tuning to modulation envelopes there. The VNLL may give rise directly to the band-pass rMTFs of neurons in the inferior colliculus or may be involved in shaping them. Judging from immunocytochemical evidence, the projection from VNLL to the inferior colliculus is largely, but not exclusively, inhibitory (Riquelme et al. 2001; Saint Marie et al. 1997). The neurons in the VNLL with band-pass MTFs could correspond to the presumed excitatory neurons in the VNLL and provide a direct excitatory projection to the inferior colliculus. In this way, band-pass tuning of neurons in the VNLL would be directly reflected in the responses of neurons in the inferior colliculus. If this is the case, neurons in the VNLL and inferior colliculus should be tuned to similar modulation frequencies. A number of studies have examined tuning to modulation frequencies in the inferior colliculus (Condon et al. 1996; Krishna and Semple 2000; Langner and Schreiner 1988; Müller-Preuss et al. 1994), but they disagree on the range of modulation frequencies to which neurons are tuned, making any comparison difficult.

A second possibility is that the band-pass tuning in the VNLL is not directly reflected in the inferior colliculus, but shapes the responses of neurons there. This could happen in much the same way that tuning to interaural intensity level differences is shaped in the inferior colliculus of the barn owl (Adolphs 1993). Such a role was suggested by Langner et al. (2006).

Comparison with other species

The present results are in general agreement with those in the VNLL of other species. The VNLL of the rat contains neurons with sustained and onset responses to short tone bursts (Zhang and Kelly 2006). A preliminary report indicates that band-pass rMTFs are present in the VNLL, but are associated with both neurons that have sustained and those that have onset responses (Zhang and Kelly 2005). Thus the proportion of neurons with band-pass tuning is much higher. Those neurons with onset responses to tones exhibit high synchrony to SAM tones, much as in the rabbit. In the bat Eptesicus, there are also neurons with band-pass rMTFs (Huffman et al. 1998). Oddly, neurons in the columnar division of the VNLL in Eptesicus do not follow SAM tones, even though these neurons are known for their precisely timed onset responses to tone bursts (Covey and Casseday 1991). In the VNLL of the rabbit, there are neurons that respond only at the onset of SAM tones, but it is unclear whether these correspond to the columnar neurons in Eptesicus. Neurons with band-pass rMTFs in the VNLL of Eptesicus appear to lie in the multipolar cell division. Neurons in this division also synchronized strongly to SAM tones.

In summary, the pathway from the cochlear nucleus to the inferior colliculus through the VNLL may be the route or one of the routes along which a rate-based tuning to modulation frequency is constructed. This pathway may contribute to our perception of the pitch of complex sounds or to our different perceptions of AM over different ranges of modulation frequency.

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