Responses of Neurons in Chinchilla Auditory Cortex to Frequency-Modulated Tones

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Submitted 18 August 2008; accepted in final form 9 February 2009

Brown TA, Harrison RV. Responses of neurons in chinchilla auditory cortex to frequency-modulated tones. J Neurophysiol 101: 2017–2029, 2009. First published February 11, 2009; doi:10.1152/jn.90931.2008. Frequency-modulated (FM) stimuli have been used to explore the behavior of neurons in the auditory cortex of several animal models; however, the properties of FM-sensitive auditory cortical neurons in the chinchilla are still unknown. Single-unit responses to FM stimulation were obtained from the auditory cortex of anesthetized adult chinchillas (Chinchilla laniger). Upward and downward linear FM sweeps spanning frequencies from 0.1 to 20 kHz were presented at speeds of 0.05 to 0.82 kHz/ms. Results indicated that >90% of sampled neurons were responsive to FM sweeps. The population preference was for upward FM sweeps and for medium to fast speeds (≥0.3 kHz/ms). Few units (3%) were selective for downward FM sweeps, whereas <22% of units preferred slow speeds (<0.1 kHz/ms). Velocity preference and direction sensitivity were positively correlated for upward sweeps only (r = 0.40, P = 0.0021, t-test).

Three types of firing rate patterns were observed in the FM response peristimulus time histograms: a single peak at sweep onset/offset (“onset”) and a single peak (“late”) or multiple peaks (“burst”) during the sweep. “Late” units expressed the highest mean values for direction sensitivity and speed selectivity; “onset” units were selective only for direction and “burst” units were not selective for either direction or speed. The robust responsiveness of these neurons to FM sweeps suggests a functional role for FM detection such as the identification of FM sweeps present in vocalizations of other organisms within the chinchilla’s natural environment.

INTRODUCTION

The auditory system has evolved to provide a representation of the external acoustic environment within the brain. Although simple tones have been the primary stimulus used to characterize the auditory cortex (e.g., Merzenich et al. 1975), it is necessary to evaluate the auditory cortical response to stimuli that are more complex yet whose properties are still easy to classify and manipulate. Frequency-modulated (FM) waveforms are composed of frequencies that change with respect to time and can be used to investigate mechanisms that are not readily apparent in responses produced by pure tones (Nelken and Versnel 2000).

A variety of studies using FM stimuli have demonstrated the presence of neurons along the auditory pathway that are sensitive to particular characteristics of FM sweeps such as sweep direction and rate of change of frequency. Tuning for FM sweep speed and direction in the auditory system is comparable to tuning for movement direction and speed in the visual system (Rauschecker 1998). FM tuning is present at lower stages in the auditory pathway with some direction selectivity but no speed sensitivity evident in auditory-nerve fibers (Sinek and Geisler 1981) and the cochlear nucleus (Britt and Starr 1976; Suga 1964). However, neurons at higher levels in the auditory pathway, including the auditory cortex, demonstrate preferences for both FM sweep speed and direction in a variety of animal models including primates (Godley et al. 2005), bats (Shannon-Hartman et al. 1992; Suga 1965), cats (Heil et al. 1992; Mendelson and Cynader 1985; Whitfield and Evans 1965), rats (Gaese and Ostwald 1995; Ricketts et al. 1998; Zhang et al. 2003), and ferrets (Nelken and Versnel 2000; Shamma et al. 1993). The above-cited studies focused mainly on the responses of neurons in primary auditory cortex (AI), whereas other studies have also found FM-responsive neurons in secondary areas such as anterior auditory fields (AAFs) in the cat (Tian and Rauschecker 1994) and ferret (Tian and Rauschecker 1998) and posterior auditory fields (PAFs) in the cat (Heil and Irvine 1998; Tian and Rauschecker 1994).

Although FM stimuli have been used to explore the behavior of neurons in the auditory cortex of a variety of animal models, the properties of FM-sensitive auditory cortical neurons in the chinchilla are still unknown. The chinchilla has been used extensively as an animal model in auditory neuroscience in part due to its good low-frequency sensitivity, which is similar to that of humans (Heffner and Heffner 1991). Extensive work has been performed in the chinchilla animal model using tone stimuli to characterize neurons in both primary and secondary auditory areas (Harel et al. 2000; Harrison et al. 1991; Pienkowski and Harrison 2005). However, characterization of neural responses to more complex stimuli such as FM sounds has not been investigated. The purpose of this study therefore was to characterize the responses in neurons of the chinchilla auditory cortex to a range of FM stimuli.

METHODS

Surgical preparation

Five adult female chinchillas (Chinchilla laniger) (weight: 540–770 g) were used in the present study (Reidstra and Sons Chinchillas, Norval, Ontario, Canada). All experimental procedures adhered to the guidelines specified by the Canadian Council on Animal Care. Chinchillas were anesthetized with ketamine (15 mg/kg, administered intraperitoneally [ip]) and xylazine (2.5 mg/kg, ip) and supplemental...
doses were administered throughout the experiment as required to
maintain a surgical level of anesthesia. Foot and tail withdrawal
reflexes were monitored throughout the experiment to control the
depth of anesthesia. Body temperature was maintained at 37°C.
Auditory brain stem responses to pure tones (0.25–8 kHz) were
measured on the right mastoid using the Intelligent Hearing Systems
Smart-EP system to verify normal levels of hearing in each animal. A
tracheotomy was performed first to provide proper ventilation so that
the head could be securely clamped at the snout to a head holder for
the duration of the experiment. Next, a craniotomy was performed to
expose the left auditory cortex for electrophysiological single-unit
recordings. The location of the auditory cortical area was determined
by using the coronal suture, the orbit wall, and the zygomatic arch as
surgical landmarks and the middle temporal artery as a surface
vasculature landmark (Panesar et al. 2001). A digital image was taken
of the exposed cortex to record the location of each microelectrode
penetration. Experiments were typically 8 to 12 h in length and the
animals were decapitated at the end of the experiment.

Electrophysiology

The activity of auditory cortical neurons was recorded using tung-
sten microelectrodes (2- to 4-MΩ impedance) at depths ranging from
200 to 800 μm relative to the cortical surface corresponding to layers
II–IV in chinchilla auditory cortex (Pienkowski and Harrison 2005).
Neural signals were amplified (×10,000–50,000), filtered (band-pass:
0.3–7 kHz), then sampled at 25 kHz. Raw data were recorded using
BrainWare (Tucker-Davis Technologies [TDT]) then stored in binary
format for further analysis using custom tools developed in MATLAB
7.0 (The MathWorks).

Single-unit discrimination

Neural activity was further analyzed to distinguish single-unit
activity from the recorded multunit data. The power of the sampled
waveforms was calculated using a 1-ms sliding window (Bankman et
al. 1993). The baseline mean and SD of the filtered waveform were
then calculated. Spikes were classified as any event that exceeded a
waveform threshold set at the baseline mean + 5 SDs (Csicsvari et al.
1998; Rutishauser et al. 2006). Principal-component analysis (PCA)
was used to reduce the multidimensional spike data sets to lower
dimensions for further analysis (Abeles and Goldstein 1977). The
PCA generated principal-component scores that represent the spike
event data set in the principal-component space. Spike clusters were
constructed by applying an “average” clustering algorithm (un-
weighted average distance between objects in a group of clusters) to
the scores of the first three dimensions of the spike data in principal-
component space. To confirm that spikes within a given cluster were
from the same unit, interspike interval (ISI) autocorrelations were
performed on the entire cluster. Valid single-unit clusters were des-
ignated as those with an ISI autocorrelation of approximately zero for
intervals <3 ms (Gerstein and Kiang 1960; Perkel et al. 1967).

Stimulus generation

Stimuli were produced by a high-frequency transducer (Intelligent
Hearing Systems) then transmitted by a tube to the right ear while
recordings were made from the left auditory cortex. The tube was
connected to a foam ear tip probe and held securely within the ear
canal. Tone stimuli were generated with SigGen Signal Generator
(TDT). Each stimulus set was calibrated by connecting the output
transducer in series with a small-volume tube cavity to a 1/2-in.
 microphone (Aco Pacific). The microphone was connected to a
spectrum analyzer (SR760 FFT, Stanford Research Systems) and the
entire stimulus set was presented and calibrated accordingly. A
frequency-response curve was generated using the measured output of
tone stimuli and a compensatory filter was created. Tone stimuli were
calibrated by adjusting the gain at each particular stimulus frequency.
FM stimuli were generated with MATLAB then calibrated by applying
the compensatory filter to each FM stimulus.

Tones

To characterize the basic response properties of each neuron (e.g.,
characteristic frequency [CF] and frequency-response area [FRA]),
animals were stimulated with tones ranging in frequency from 0.4 to
20 kHz (50-ms duration with a 5-ms rise/fall time) in 1/3-octave steps
at intensities of 20, 40, and 60 dB SPL. Stimuli were presented in a
pseudorandom manner of frequency and intensity combinations at a
rate of one stimulus every 500 ms for 30 repetitions. Responses were
recorded from 50 ms prior to stimulus onset to 50 ms after stimulus
offset (duration of 150 ms).

FM stimuli

Animals were stimulated with FM signals sweeping linearly upward
and downward through a fixed range of 0.1 to 20 kHz to
maximally encompass the FRAs of sampled neurons. FM sweep
speeds ranged from 0.05 to 0.82 kHz/ms in log steps at an intensity of
60 dB SPL. Upward-sweep stimuli consisted of 150 ms at the low
frequency of 0.1 kHz followed by an upward FM sweep (variable
duration depending on FM sweep speed) from 0.1 to 20 kHz followed
by a variable duration at the high frequency of 20 kHz. Downward-
sweep stimuli were constructed in a similar manner. The entire
stimulus duration was 900 ms. Stimuli were presented in a pseudo-
random manner of sweep direction and speed combinations at a rate
of one stimulus every 1,500 ms for 30 repetitions. Responses were
recorded from 50 ms prior to stimulus onset to 50 ms after stimulus
offset (duration of 1 s).

Data analysis

Criteria commonly reported in the auditory literature were used to
measure the neuronal responses to tones and FM sweeps because this
provided a basis for appropriate comparisons with previous studies.
Statistical analysis was performed using MATLAB and Sigma Plot
(Systat Software). Student’s t-test, one-way ANOVA, and Tukey’s
honestly significant difference (HSD) criteria were used to measure
significance among group means. A level of significance of P < 0.05
was used throughout the analysis. Mean values are presented as
mean ± SE.

Analysis of responses to tones

Responses to tones were grouped into peristimulus time histograms
(PSTHs) for each stimulus frequency using 5-ms time bins averaged
over 30 presentations to produce mean firing rates (spikes/s) (see Fig. 1C
for an example). The spontaneous rate was obtained from the 50-ms
period before stimulus onset for all stimulus frequencies and a mean
rate was determined. The driven firing rate was calculated as the mean
spontaneous rate subtracted from the firing rate in each bin. A re-
sponse was present if the mean driven firing rate was >2 SDs of the
spontaneous rate. The best frequency of a unit at a given intensity was
defined as the frequency at which the maximum peak occurred in the
frequency-response curve. The CF for a unit was defined as the best
frequency at the lowest intensity that elicited a response. The tuning
bandwidth of the unit was defined as the span of frequencies (in
octaves) over which the mean driven firing rate remained >50% of
the peak rate in the frequency-response curve.

Analysis of responses to FM sweeps

Responses to FM sweeps were grouped into PSTHs for each speed-
direction pairing using 5-ms time bins averaged over 30 presentations to
produce mean firing rates (spikes/s) (see Fig. 1A for an example). The response period was defined as the period from sweep onset to 100 ms after sweep offset. The mean spontaneous rate was calculated in a similar manner as previously described for responses to tones. The driven firing rate was obtained by subtracting the mean spontaneous rate from the firing rate in each bin in the PSTH. Local maxima in the PSTH were classified as peaks if the local maximum during the response period was >3 SDs of the spontaneous rate. If multiple local maxima were present, each was classified as distinct only if the local minimum between each pair of local maxima was <3 SDs of the spontaneous rate. A unit was considered for analysis only if responses were present in at least two of the five PSTHs in each sweep direction.

Variations on FM response measurements described in the literature (Godey et al. 2005; Mendelson and Cynader 1985; Nelken and Versnel 2000) were used to assess the relationship between unit response and FM sweep speed and direction (direction sensitivity, velocity sensitivity, and speed tuning). Briefly, each peak in each PSTH was fitted with a Gaussian curve using a least-squares approximation (Levenberg–Marquardt optimization algorithm). The total area under each Gaussian curve was used as the unit response parameter for the corresponding sweep speed and direction in the FM response measurement calculations.

The direction sensitivity (DS) of a unit was defined as the sum of the response areas in the upward sweep direction minus the sum of the response areas in the downward sweep direction, divided by the total sum of response areas in the upward and downward directions. The resulting value lies between $-1$ and $+1$

$$DS = \frac{(\sum \text{Area} \uparrow - \sum \text{Area} \downarrow)}{(\sum \text{Area} \uparrow + \sum \text{Area} \downarrow)}$$

Units with direction sensitivities approaching ±1 have a strong preference for sweep direction. Units with direction sensitivities >0.3 are classified as upward selective, whereas units with direction sensitivities less than −0.3 are downward selective. Units with direction sensitivities that fall between these thresholds are classified as non-selective for direction.

The velocity sensitivity (VS) of a unit was defined as the sum of the product of each response area and its corresponding sweep speed.
divided by the total sum of response areas, and was calculated for each sweep direction. The velocity sensitivity value lies on a continuum between the lowest and highest presented sweep speeds

\[ VS = \frac{\sum (Area \times Speed)}{\sum Area} \]

The speed tuning (ST) of a unit was defined as one minus the ratio of the average response area and the maximum response area and was calculated for each sweep direction. A coefficient (the number of sweep speeds divided by the number of speeds minus one) is applied so that all ST values lie in the range of 0 to 1. A value of 0 implies no selectivity for a single speed and a value of 1 implies that the unit is selective for a single speed

\[ ST = \frac{5}{4} \times \left[ 1 - \frac{\text{mean (Area)}}{\text{maximum (Area)}} \right] \]

A threshold value of 0.625 is used to qualify the selectivity of each unit. At this threshold, the area of the maximum response is twice as large as the mean of all response areas. Units with ST values above this threshold are classified as highly selective or narrowly tuned, whereas units that fall below this threshold are described as poorly selective or broadly tuned.

RESULTS

Neural responses to FM sweeps

Responses were recorded from 142 units in the auditory cortex. Units were generally responsive to FM sweeps. An example of a unit that demonstrated strong speed and direction selectivity is illustrated in Fig. 1. As shown in Fig. 1A, this unit responded more robustly to upward (left column) than to downward sweeps (right column), with the strongest response present at 0.41 kHz/ms. This unit exhibited a strong preference for upward-sweep direction, as suggested by a direction sensitivity of 0.95 as well as narrow speed tuning (0.67) for high speeds (velocity sensitivity: 0.54 kHz/ms) in this direction. Narrow tuning (0.75) for high speeds (velocity sensitivity: −0.54 kHz/ms) was also evident in the nonpreferred (downward) sweep direction. In contrast, an example of a unit that did not demonstrate a strong preference for direction or speed is displayed in Fig. 7. As depicted in Fig. 7A, this unit responded robustly to sweeps in both directions at all speeds with maximum responses at an upward-sweep speed of 0.41 kHz/ms and a downward-sweep speed of −0.10 kHz/ms. This unit exhibited poor direction sensitivity (0.01) and weak speed tuning (0.42 for upward sweeps and 0.17 for downward sweeps). Multiple peaks were observed in the PSTH for both upward and downward sweeps at speeds of ≤0.20 kHz/ms in each direction.

FM response parameters

Population values of direction sensitivity, velocity sensitivity, and speed tuning for sampled units are illustrated in Fig. 2.

FIG. 2. Population distributions of FM response parameters for FM-responsive units. A: direction sensitivity. The majority of units (88%) had positive direction sensitivities with a median value of 0.55. The overall population preference is for upward sweeps. B: velocity sensitivity. Black bars represent distribution for upward sweeps. Light bars represent distribution for downward sweeps. Units exhibited similar absolute median velocity sensitivity values for both sweep directions (upward sweeps: 0.38 kHz/ms; downward sweeps: 0.33 kHz/ms). C: speed tuning. Units exhibited stronger speed tuning for downward sweeps (median = 0.68) than that for upward sweeps (median = 0.54). n = 142 units.
ward-selective units comprised 3%. Figure 2B illustrates the distribution of velocity sensitivities in each FM sweep direction, with dark bars representing the population distribution during upward sweeps and the light bars representing the distribution during downward sweeps. Units demonstrated absolute mean velocity sensitivities that lay in the middle of the linear range of presented speeds (upward sweeps: $0.39 \pm 0.01$ kHz/ms; downward sweeps: $-0.35 \pm 0.01$ kHz/ms). Although the magnitude of mean velocity sensitivities were similar for sweeps in both directions, Fig. 2C shows that units demonstrated narrow mean speed tuning ($0.65 \pm 0.02$) for downward sweeps (light bars) and broader mean speed tuning ($0.56 \pm 0.02$) for upward sweeps (dark bars). More than half (58%) of sampled units were narrowly tuned for speed during downward sweeps, 39% were narrowly tuned during upward sweeps, and 30% were narrowly tuned during sweeps in both directions.

The relationships between FM response parameters are illustrated in Fig. 3. Velocity sensitivity exhibited a positive correlation with speed tuning in both sweep directions as shown for upward sweeps in Fig. 3C ($r = 0.57$, $P < 0.0001$, $t$-test) and as illustrated more modestly for downward sweeps in Fig. 3F ($r = 0.18$, $P = 0.0320$, $t$-test). Direction sensitivity was indicated a statistically significant regression slope. Significance level: $P < 0.05$.

FIG. 3. Relationships between FM response parameters for FM-responsive units for upward (left column) and downward sweeps (right column). Solid line in each plot is the regression line. A and D: direction sensitivity vs. velocity sensitivity. B and E: direction sensitivity vs. speed tuning. C and F: speed tuning vs. velocity sensitivity. $y = $ equation of regression line; $r = $ correlation coefficient; $n = $ number of units. * indicates a statistically significant regression slope. Significance level: $P < 0.05$. 

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was correlated with velocity sensitivity (Fig. 3D: \( r = 0.20, P = 0.0198, t\)-test) and speed tuning (Fig. 3E: \( r = 0.24, P = 0.0034, t\)-test) for downward sweeps but was correlated only with velocity sensitivity for upward sweeps (Fig. 3A: \( r = 0.37, P < 0.0001, t\)-test). These regression results suggest that units which displayed a preference for downward sweeps were more likely to prefer slow speeds while upward-selective units were more likely to prefer fast sweep velocities. The results also imply that units which were highly selective for speed also preferred fast sweep speeds. There was no evidence of an orderly distribution of the aforementioned FM response parameters across the surface of the auditory cortex.

Figure 4 illustrates the population distributions and parameter relationships for units that were narrowly tuned for speed. In Fig. 4A, the distribution of direction sensitivities of speed-selective units indicated an overall preference for upward sweeps (mean: 0.53 ± 0.04). The distribution of velocity sensitivities, shown in Fig. 4B, demonstrated that units that were speed-selective during upward sweeps expressed higher absolute median velocity sensitivity (dark bars: 0.53 kHz/ms) than those that were selective for speed during downward sweeps (light bars: −0.37 kHz/ms). While velocity sensitivity was correlated with direction sensitivity for units which expressed speed selectivity during upward sweeps (Fig. 4C: \( r = 0.40, P = 0.0021, t\)-test), no relationship was evident between these parameters for speed-selective units during downward sweeps (Fig. 4D).

**Temporal patterns of neural responses to FM sweeps**

Distinct patterns were observed in the temporal responses of sampled units to FM sweeps. These firing rate patterns were similar to those previously described in the literature (Whitfield and Evans 1965). Single-peak units exhibited a single dominant peak in their PSTHs. This group was further subdivided into onset-response and late-response units. Figure 5 illustrates an example of a typical onset-response unit. In Fig. 5A, the responses of this unit to FM sweeps were characterized by a peak occurring at or after sweep onset for upward sweeps (left column) and at or after sweep offset for downward sweeps (right column). Note that the peak response latency for upward sweeps was consistent across all sweep speeds. Additionally, note that the peak in each downward sweep PSTH occurred after the end of the FM sweep presentation represented by the solid black bar beneath the PSTH. All other single-peak units were classified as late-response units. Figure 6 illustrates a typical late-response unit. In Fig. 6A, this unit’s responses are characterized by a peak occurring after sweep onset for upward sweeps and before sweep offset for downward sweeps. Note that the peak response latency for both sweep directions decreased with increasing sweep speed and that the
peak in each PSTH occurred during presentation of the FM sweep. Burst-response units exhibited multiple distinct peaks in their PSTH. Figure 7 presents a typical example of a burst-response unit. As demonstrated in Fig. 7A, the distance between the peaks within each PSTH decreased with increasing sweep speed for sweeps in both directions, whereas the response latency of the first peak in each PSTH also decreased with increasing sweep speed (compare responses at 0.05 kHz/ms with those at 0.10 and 0.20 kHz/ms).

From the sample of 142 FM-responsive units, 42 were classified as onset-response, 68 as late-response, and 32 as burst-response units. Mean values for direction sensitivity, velocity sensitivity, and speed tuning for each response type are recorded in Table 1. All response types demonstrated mean velocity sensitivities between 0.33 and 0.44 kHz/ms. Late-response units had the largest overall mean direction sensitivity (0.63 ± 0.04, \(P < 0.0001\), Tukey’s HSD). Although 85% of this group expressed direction selectivity, all but one of these direction-selective units preferred upward sweeps. These units also expressed the highest mean velocity sensitivity (0.44 ± 0.02, \(P = 0.0001\), Tukey’s HSD) and mean speed tuning (0.62 ± 0.02, \(P = 0.0007\), Tukey’s HSD) during the presentation of upward sweeps. More than half (56%) of late-response units were narrowly tuned for speed during upward sweeps (velocity sensitivity = 0.52 ± 0.03 kHz/ms), whereas 72% expressed speed selectivity during downward sweeps (velocity sensitivity = −0.38 ± 0.03 kHz/ms). Onset-response units had a mean direction sensitivity of 0.39 ± 0.08 and a greater mean speed tuning during downward sweeps (0.68 ± 0.03) than during upward sweeps (0.51 ± 0.03). Like late-response units, most onset-response units (81%) expressed direction selectivity with only 12% of these direction-selective units preferring downward sweeps. The majority of onset units (60%) was narrowly tuned for speed during upward sweeps (velocity sensitivity = −0.35 ± 0.04 kHz/ms) and only 29% of onset units were selective for speed during upward sweeps.
(velocity sensitivity $= 0.40 \pm 0.05$ kHz/ms). As a population, burst-response units were not selective either for direction (direction sensitivity $= 0.29 \pm 0.05$) or for speed (mean speed tuning $\leq 0.50$ in both directions). However, half of burst-response units expressed direction selectivity and all these direction-selective units preferred upward sweeps. Only a small portion of burst units expressed speed selectivity in either sweep direction: 19% were selective for speed during upward sweeps with a mean velocity sensitivity of $0.37 \pm 0.07$ kHz/ms and 28% were speed-selective during downward sweeps with a mean velocity sensitivity of $-0.38 \pm 0.07$ kHz/ms.

Neural responses to tones

Responses to both FM and tone stimuli were recorded from 116 units. Figure 1 illustrates the tone responses for an FM-responsive unit. The frequency-response curves obtained from tones presented at 20, 40, and 60 dB SPL are presented in Fig. 1B (top row). This unit had a response threshold of 40 dB, a CF of 6.04 kHz (indicated by the arrows in the frequency-response curve plots at 40 and 60 dB SPL), a best frequency of 8.06 kHz at 60 dB SPL, and tuning bandwidths of 1.33 and 2.33 octaves at 40 and 60 dB SPL, respectively. In the frequency-response curve at 60 dB, this unit displayed a single region where the driven firing rate remained $50\%$ of the firing rate at the best frequency. Within the sample population, 48% of units displayed similar single-peak characteristics in their frequency-response curves at 60 dB, whereas 21% displayed two peaks and the remaining 31% exhibited three or more peaks. The occurrence of spike events in the raster plots presented in Fig. 1B (bottom row) was representative of the majority of sampled units. In this example, the unit responded robustly to tones between 3.2 and 16 kHz shortly after tone onset and ceased to respond after tone offset. Figure 1C illustrates the phasic nature of these responses.
as demonstrated by the dominant single peaks in the PSTH of responses to tones within the FRA. Note that the responses for this unit peak during the presentation of each tone represented by the solid black bar beneath each PSTH and diminish before the offset of the tone. No units responding solely to tone offset were observed within the sample.

**Tone response parameters**

Population distributions of CFs, minimum first-spike latencies, and tuning bandwidths at 60 dB SPL are displayed in Fig. 8. Units with CF between 0 and 10 kHz represented 80% of the population, whereas 20% of units had higher CF (Fig. 8A). The horizontal bar below each PSTH represents the duration of the pure tone. This unit’s FRA spanned frequencies from 3.2 to 16.1 kHz.

**TABLE 1. Population values for FM response parameters of FM-responsive units with respect to FM-response types**

<table>
<thead>
<tr>
<th>Response Type</th>
<th>DS</th>
<th>VS, kHz/ms</th>
<th>ST</th>
<th>VS, kHz/ms</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>0.39 ± 0.08</td>
<td>0.36 ± 0.01</td>
<td>0.51 ± 0.03</td>
<td>0.35 ± 0.03</td>
<td>0.68 ± 0.03</td>
</tr>
<tr>
<td>Late</td>
<td>0.63 ± 0.04*</td>
<td>0.44 ± 0.02*</td>
<td>0.62 ± 0.02*</td>
<td>0.36 ± 0.02</td>
<td>0.71 ± 0.02</td>
</tr>
<tr>
<td>Burst</td>
<td>0.29 ± 0.05</td>
<td>0.53 ± 0.02</td>
<td>0.47 ± 0.03</td>
<td>0.34 ± 0.02</td>
<td>0.49 ± 0.04*</td>
</tr>
</tbody>
</table>

Values are means ± SE. DS, direction sensitivity; VS, velocity sensitivity; ST, speed tuning. Results of Tukey’s HSD tests for each FM response parameter: direction sensitivity (P < 0.0001), velocity sensitivity (upward sweeps: P = 0.0001; downward sweeps: P = 0.9083); speed tuning (upward sweeps: P = 0.0007; downward sweeps: P < 0.0001). Onset: n = 42 units; late: n = 68 units; burst: n = 32 units. * indicates means that are significantly different from the group. Significance level: P < 0.05.

FIG. 7. Responses of a characteristic burst-response unit to FM sweeps and pure tones. A: PSTHs of responses to upward and downward FM sweeps at 60 dB SPL. The horizontal bar below each PSTH represents the duration of the FM sweep. This unit exhibited multiple distinct peaks in the PSTH for both upward and downward sweeps (direction sensitivity = 0.01; upward sweeps: velocity sensitivity = 0.26, speed tuning = 0.42; downward sweeps: velocity sensitivity = 0.30, speed tuning = 0.17). B: frequency-response curves (top row) and raster plots of spike occurrence times (bottom row) in response to tones at 20, 40, and 60 dB SPL. Vertical lines in the raster plots indicate stimulus onset and offset times. The arrows in the frequency-response curves indicate the CF of this unit (8.06 kHz). C: PSTH of responses at each of the frequencies within the unit’s FRA at 60 dB SPL. The horizontal bar below each PSTH represents the duration of the pure tone. This unit’s FRA spanned frequencies from 3.2 to 16.1 kHz.
Best frequencies at 60 dB SPL were distributed with 59% below 5 kHz, 22% between 5 and 10 kHz, and 19% above 10 kHz. Figure 8 presents the distribution of first-spike latencies, which was centered at 15.06 ms with a mean of 15.59 ± 0.33 ms. The sampled units exhibited a median tuning bandwidth of 2.33 octaves and a mean of 2.68 ± 0.12 octaves, as shown in Fig. 8C.

Relationships between tone and FM response parameters

There was some evidence of modest relationships between FM and tone response parameters as summarized in Table 2. First spike latency was negatively correlated with velocity sensitivity for both upward ($r = -0.24, P = 0.0087, t$-test) and downward ($r = -0.26, P = 0.0058, t$-test) sweeps. First spike latency was also negatively correlated with velocity sensitivity for speed-selective units (upward sweeps: $r = -0.37, P = 0.0135, t$-test; downward sweeps: $r = -0.33, P = 0.0049, t$-test). Tuning bandwidth expressed a negative correlation with speed tuning in both sweep directions (upward sweeps: $r = -0.23, P = 0.0123, t$-test; downward sweeps: $r = -0.28, P = 0.0025, t$-test). CF was positively correlated with velocity sensitivity for upward ($r = 0.31, P = 0.0007, t$-test) and downward sweeps ($r = 0.29, P = 0.0019, t$-test) as well as with speed tuning ($r = 0.27, P = 0.0040, t$-test) but only during the presentation of upward sweeps. CF was also correlated with velocity sensitivity for speed-selective units but only for those which expressed narrow speed tuning during upward sweeps ($r = 0.27, P = 0.0040, t$-test). These correlation coefficients suggest that units which displayed fast velocity sensitivities were associated with higher CF and shorter first spike latencies. An additional implication is that units with narrow tuning bandwidths were more likely to have better speed tuning than units with broader tuning bandwidths.

Table 2. Correlation coefficients of tone–FM response parameter pairs for FM-responsive units at 60 dB SPL

<table>
<thead>
<tr>
<th>FM Sweeps</th>
<th>Latency, ms</th>
<th>BW, octaves</th>
<th>CF, kHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS ↑, kHz/ms</td>
<td>-0.13</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>VS ↓, kHz/ms</td>
<td>-0.24*</td>
<td>-0.16</td>
<td>0.31*</td>
</tr>
<tr>
<td>n-ST VS ↑, kHz/ms</td>
<td>-0.26*</td>
<td>0.04</td>
<td>0.29*</td>
</tr>
<tr>
<td>n-ST VS ↓, kHz/ms</td>
<td>-0.37*</td>
<td>0.14</td>
<td>0.23</td>
</tr>
<tr>
<td>ST ↑</td>
<td>-0.33*</td>
<td>0.06</td>
<td>0.30*</td>
</tr>
<tr>
<td>ST ↓</td>
<td>0.07</td>
<td>-0.23*</td>
<td>0.27*</td>
</tr>
<tr>
<td>CF</td>
<td>0.09</td>
<td>-0.28*</td>
<td>0.03</td>
</tr>
</tbody>
</table>

DS, direction sensitivity; VS, velocity sensitivity; n-ST VS, velocity sensitivity for units with narrow speed tuning; ST, speed tuning; Latency, first-spike latency; BW, tuning bandwidth; CF, characteristic frequency; ↑, upward sweeps; ↓, downward sweeps. * indicates a statistically significant regression slope. Significance level: $P < 0.05$. 

Tone responses of each FM temporal response type

When tone responses were sorted by FM firing rate response type, several patterns emerged. Figure 9 illustrates how the distribution of CFs varied for each FM response type. Units with low-frequency CF (<5 kHz) comprised 53% of the total sample. Within this group, 35 units exhibited onset-response behavior, 17 were classified as late-response onset units, and 9 units were described as burst-response types. For midrange CF (5 to 10 kHz), 20 late-response units, 11 burst-response units, and only one unit with onset-response tendencies were observed. At higher frequencies (>10 kHz), late-response behavior was attributed to 14 of the 23 units, whereas 8 were burst-response units and only one onset-response unit was present.
Almost all onset-response units (95%) responded best to low frequencies. Figure 5 illustrates the tone responses of a typical onset unit. As shown in Fig. 5B (top row), the CF of this unit was 0.4 kHz and is indicated by the arrows in the frequency-response curves. Since the CF was found at the lowest tone frequency presented, it is quite possible that the actual CF of this unit may be even lower than the value represented here. This unit exhibited a single dominant peak in its frequency-response curve at 60 dB and reflects the behavior of 40% of all onset-response units. Characteristic frequencies were more evenly distributed for late-response units, with 33% expressing low-frequency CF, 39% expressing medium-frequency CF, and 28% expressing high-frequency CF. Figure 6 illustrates an example of a late-response unit with a high CF. This unit demonstrated a FRA from 8.06 to 16.1 kHz at 60 dB and a CF of 16.1 kHz, indicated by the arrows in the frequency-response curves of Fig. 6B (top row). Like the onset unit described earlier, this unit exhibited a single dominant peak in its frequency-response curve at 60 dB. The majority of late-response units (52%) also exhibited a single dominant peak in their frequency-response curves at 60 dB. The CFs of burst-response units were distributed in a similar manner to that of late-response units, with 32% in the low-frequency range, 39% in the 5- to 10-kHz range, and 29% classified as high-frequency units. Figure 7 illustrates the tone responses of a typical burst-response unit. This unit had a midrange CF of 8.06 kHz indicated by the arrows in the frequency-response curves illustrated in Fig. 7B (top row). Although this unit displayed multiple distinct peaks in its frequency-response curve at 20 dB, the distinctions between peaks disappeared with increasing stimulus intensity thus merging the peaks into a single region in the frequency-response curve obtained at 60 dB. This single-peak attribute was also observed in the frequency-response curves of 50% of burst units at 60 dB SPL. The response PSTHs obtained for tones within the FRA of each of the previously described FM-response units were not dramatically different in their temporal patterns (Figs. 4C, 5C, and 6C). Mean tone response parameter values for each response type are listed in Table 3. Burst-response units exhibited the shortest overall first-spike latency (14.19 ± 0.54 ms, P = 0.0019, Tukey’s HSD) and the broadest tuning bandwidth (3.28 ± 0.22 octaves).

**DISCUSSION**

**FM response parameters**

FM-responsive units expressed general preferences for upward sweeps and were selective for fast speeds in this direction. The preference for upward sweeps in the chinchilla model is in contrast with results from squirrel monkey (Godey et al. 2005) and owl monkey AI (Atencio et al. 2007), where the mean direction sensitivities reported in both studies were close to zero, thus suggesting that, as a group, monkey AI units did not exhibit a preference for sweep direction. Although the overall best speed was in the middle of the sweep rates presented, the speed tuning of the population of units was broad to moderate. The differences in these results could be attributed to the environmental significance of the FM sweep speeds used in both studies. In the primate studies, the presented FM speeds fell within the range of monkey twitter vocalizations, whereas the lowest FM sweep speeds used in the current study were an order of magnitude faster than those observed in samples of chinchilla vocalizations.

The proportion of direction-sensitive units reported here is similar to the proportion reported in cat primary auditory cortex (Mendelson and Cynader 1985). The majority of direction-sensitive units in that study also preferred upward sweeps, although the percentage reported in the present study was greater by 30%. Although these results are in contrast with those from Heil et al. (1992), where 70% of units in cat AI were nonselective for direction and the majority of selective units preferred downward sweeps, the majority of FM direction-selective units in rat AI (Orduna et al. 2001; Ricketts et al. 1998), and secondary areas such as cat AAF (Tian and Rauschecker 1994) and PAF (Tian and Rauschecker 1998) preferred upward sweeps. Similarly, the preferred speeds in both cat and rat models were in the medium and fast ranges of presented speeds.

Several mechanisms have been proposed to explain the directional asymmetry observed in FM-responsive units. One such mechanism involves the extent to which the FM sweep range overlaps the FRA and the direction of modulation relative to the unit’s best frequency (Phillips et al. 1985). In the present study, some units exhibited the relationship between direction sensitivity and FRA suggested by this mechanism (i.e., high direction sensitivity for units with BF at the extremes of the FM sweep range and low direction sensitivity for units with BF near the center of the sweep range). However, there were examples of units with frequency-response curves that lay entirely within the FM sweep range, yet that demonstrated a

**TABLE 3. Population values for tone response parameters of FM-responsive units with respect to FM response types at 60 dB SPL**

<table>
<thead>
<tr>
<th>Response Types</th>
<th>Latency, ms</th>
<th>BW, octaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset</td>
<td>17.18 ± 0.66</td>
<td>2.71 ± 0.25</td>
</tr>
<tr>
<td>Late</td>
<td>15.22 ± 0.45</td>
<td>2.32 ± 0.15#</td>
</tr>
<tr>
<td>Burst</td>
<td>14.19 ± 0.54*</td>
<td>3.28 ± 0.17#</td>
</tr>
</tbody>
</table>

Values are means ± SE. Latency, first-spike latency; BW, tuning bandwidth. Results of Tukey’s HSD tests for each tone response parameter: first-spike latency (P = 0.0041); tuning bandwidth (P = 0.1969). Onset: n = 26 units; late: n = 51 units; burst: n = 35 units. * indicates means that are significantly different from the group. # indicates pairs that have significantly different means. Significance level: P < 0.05.
marked preference for upward sweeps (e.g., the unit represented in Fig. 1). There were also examples of units with low BF and frequency-response curves that lay partially outside the lower edge of the sweep range, yet that had robust responses to sweeps in both directions (e.g., the unit represented in Fig. 5).

Although the proposed mechanism provides a suitable explanation for directional asymmetry in some of the sampled units, it does not adequately explain all the observed responses in chinchilla auditory cortex. A second mechanism relates to the presence of inhibitory and excitatory areas that flank the response areas of these units (Suga 1964). Although the inhibitory areas of sampled units were not explicitly determined, further exploration of this mechanism in future experiments may be useful in assessing the role, if any, that it plays in the direction preferences observed in chinchilla auditory cortical neurons.

In comparing responses to tones and responses to FM sweeps, some studies have reported a correlation between CF and direction sensitivity (Godey et al. 2005; Zhang et al. 2003), whereas others have not observed such a relationship (Atencio et al. 2007; Mendelson et al. 1993). Although direction sensitivity and CF were uncorrelated in the present study, it was observed that velocity sensitivity demonstrated a moderate positive correlation with CF. The correlation between velocity sensitivity and CF was also reported in cat PAF (Tian and Rauschecker 1994) and cat AAF (Tian and Rauschecker 1998), but is contrary to the observation in rat AI (Ricketts et al. 1998) where CF was independent of preferred speed.

**FM temporal response types**

Note in Fig. 5A that the peak response in the PSTH of this typical onset-response unit occurred at approximately the same time relative to sweep onset for all upward sweep speeds and to sweep offset for all downward sweep speeds. This static peak position suggests that upward sweeps passing through the FRA of this unit originate within the response area at 60 dB SPL illustrated in Fig. 5B (top row). The start frequency, end frequency, and sweep range for each sweep speed are fixed; therefore each sweep starts within the response area regardless of sweep speed and onset-response units would be stimulated immediately following sweep onset. The latency to peak response would occur at approximately the same time relative to sweep onset for all upward-sweep presentations and to sweep offset for downward sweeps independent of sweep speed. Given that the sweep starts within the FRA, the expectation would therefore be that these units would also respond to the start frequency presented prior to the FM sweep. This was indeed the case for several onset units. In other instances, however, the start frequency failed to elicit a response in the period before sweep onset. A possible explanation is that these units may have narrow response areas that lie entirely in the sweep range and low CF, which are encountered early during the sweep (for example, a sweep moving at 0.05 kHz/ms will encounter frequencies from 0.1 to 1.1 kHz within the first 20 ms). The latency to the peak response would thus appear to occur relatively close to sweep onset with low variability for all sweep speeds. The aforementioned theories are supported by the proportion of onset-response units (84%) with CF of ≤2 kHz, compared with the proportion of burst-response (7%) and late-response (16%) units. A third possibility is that these units are motion detectors that are simply responding to the changing frequencies within the sweep and not to a particular component of the sweep (Whitfield and Evans 1965). In Fig. 6A, the illustrated late-response unit exhibited the characteristic variable peak latency in the FM response PSTH, which changed with sweep speed. The response areas of late-response units appeared to be encountered earlier in each sweep as the sweep speed increased. Since the time at which an upward sweep encounters the response area changes with sweep rate, it would be expected that the latency of the peak in the PSTH would decrease as sweep speed increases and this is what was observed.

Single-peak units constituted the majority of FM-responsive cells in the present study, with almost twice as many late-response units as onset-response units observed within this group. In a study of FM responses in cat AAF (Tian and Rauschecker 1994), the majority of units displayed similar single peak behavior, whereas only 5% of these single-peak units exhibited onset-response behavior. Given that differences between the animal models and sampled areas of the auditory cortex used in that study and in the present study are important, one additional contributor to the dissimilarity in the reported number of onset-response units might be the modulation range used in each study. The FM sweeps used in the present study were modulated over a fixed range independent of unit BF and response area, whereas the cat AAF study used stimuli that encompassed a range of ±1 octave beyond each unit’s FRA, thus causing each sweep to pass through both lower- and upper-frequency boundaries of the response area.

Figure 7A depicts a typical burst unit that was characterized by multiple distinct peaks within the FM response PSTH. It is possible that burst units have multiple, excitatory regions within the FRA and that responses are thus elicited each time an upward (downward) FM sweep encounters the lower (upper) frequency boundary of each region. Like the FRA of the example burst unit at 20 dB SPL displayed in Fig. 7B (top row), half of burst units presented two or more peaks in their associated frequency-response curves, thus lending support to this theory. As the data also suggest, 50% of burst units presented a single peak in their frequency-response curves. This finding may be a result of the 1/3–octave resolution of the tone stimuli used in the present study. A finer resolution between presented tones would provide finer details about the response areas of these units and could potentially unveil additional peaks in their frequency-response curves.

The results of this study address several important questions with respect to the behavior of chinchilla auditory cortical neurons in response to complex stimuli—in this case, FM sweeps. The robust responsiveness of these neurons to FM sweeps suggests a functional role for FM detection. Since the FM stimuli used in this study were above the range of FM sweeps observed in chinchilla vocalizations, it is not yet appropriate to draw any conclusions on the role these neurons in the auditory cortex may play in chinchilla-specific communication. A worthwhile experiment would thus be to use FM sweeps with speeds that lie within the range of FM components of chinchilla vocalizations (<0.01 kHz/ms). The results of such an experiment could be compared with the results of the present study to determine whether the trend toward preferences for upward sweeps and medium to fast sweep speeds would still be a valid one and whether the environmental...
relevance of the FM stimuli would be a factor in the observed preferences. We thus suggest that the characteristics of the FM detectors observed in this study may be important in identifying FM sweeps present in vocalizations of other organisms within the chinchilla’s natural environment.

ACKNOWLEDGMENTS

We thank R. Mount for assistance in this work.

GRANTS

This work was supported by grants from the Canadian Institutes for Health Research, the Masonic Foundation of Ontario, and the Natural Sciences and Engineering Research Council.

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