Heterogeneous Neuronal Responses to Frequency-Modulated Tones in the Primary Auditory Cortex of Awake Cats

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INTRODUCTION

FM is an important feature of communication sounds, including both human speech and animal vocalizations; and the neuronal mechanisms that underlie the processing of FM signals have therefore received considerable attention. Many electrophysiological studies have been conducted on the auditory cortex of numerous species (bats: Suga 1965; rats: Mendelson and Ricketts 2001; Zhang et al. 2003; ferrets: Kowalski et al. 1995; Nelken and Versnel 1999; Shamma et al. 1993; cats: Heil and Irvine 1998; Heil et al. 1999; Mendelson and Grasse 1992; Phillips et al. 1985; Tian and Rauschecker 1994, 1998; and monkeys: Godøy et al. 2005; Tian and Rauschecker 2004). These studies in anesthetized animals generally reported that the auditory cortical neurons responded to FM sweeps in a phasic discharge pattern; that is, neuronal discharges were elicited only when the instantaneous frequency of FM sweep reached a particular trigger frequency corresponding to either the upper or lower boundary of the neuron’s tonal receptive field (TRF). All neuronal discharges were restricted to considerably shorter time intervals compared with those required for FM sweeps to traverse the TRF. Such a phasic discharge pattern conforms to the general observation that most auditory cortical neurons in anesthetized animals respond only transiently at stimulus onset (deCharms and Merzenich 1996; DeWeese et al. 2003; Eggermont 1997; Phillips 1985).

In contrast to the majority of studies on anesthetized animals, few studies of FM responses have been conducted in the awake state. Suga and his colleagues (Edamatsu et al. 1989; O’Neill and Suga 1982; Tsuzuki and Suga 1988) have conducted such experiments on awake bats and found that neuronal responses to particular aspects of FM stimuli, a crucial component of the bat’s biosonar system, are topographically organized in the auditory cortex. To date, only two studies have been conducted on common mammals lacking acoustic specialization: a pioneering study on awake cats (Whitfield and Evans 1965) and a recent study on awake owl monkeys (Atencio et al. 2007). About four decades ago, Whitfield and Evans (1965) reported the presence of multiple FM response types in cat primary auditory cortex (A1) and qualitatively categorized them into four classes: 1) the cells commenced firing as a FM tone crossed the boundary into its TRF and continued to fire until it left; 2) the cells started and ceased firing when FM tones were wholly within TRF; 3) the cells fired when the FM tone was outside TRF; 4) the cells responded to FM tones, but not to steady tones. The recent study on the A1 of awake monkeys (Atencio et al. 2007) also reported the presence of a sustained discharge mechanism responding to FM stimuli. Thus FM responses in awake A1 were more complicated than under anesthesia.

Since there are too few studies on FM responses in awake mammals (except for bats), and there are obvious differences between the results obtained from awake and anesthetized subjects, we re-examined this issue in awake cats using modern techniques. We recorded single-unit responses of A1 neurons driven by pure tone and FM sweeps in awake cats and compared the individual neuronal responses between two stimulus categories. Our results confirmed the observations of Whitfield and Evans (1965), in that we found multiple FM response patterns associated with different tonal response patterns. These distinct response patterns may provide multiple methods to fully represent FM signals, including frequency extent, sweep speed, and direction.

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METHODS

Animal preparation, recording, and histology

Experiments were performed in a manner consistent with the Guidelines for Animal Experiments, University of Yamanashi. Animal preparation and recording procedures were similar to those used in our previous experiments (Qin et al. 2005, 2007). Under pentobarbital sodium anesthesia and aseptic conditions, an aluminum cylinder (inner diameter, 12 mm) was implanted bilaterally into the temporal bone for microelectrode access. A metal block was embedded in a dental acrylic cap to immobilize the head. After 2–3 wk of postoperative recovery and adaptation training, the recording experiments started in an electrically shielded, sound-attenuated chamber. A 1- to 2-mm diam hole was drilled in the skull 1 day before each recording session under ketamine anesthesia (initial dose, 15 mg/kg). On the recording day, the dura was pierced with a sharpened probe without anesthesia, and a single epoxylite-insulated tungsten microelectrode (FHC; impedance: 2–5 MΩ at 1 kHz) was advanced into the A1 using a remote-controlled micromanipulator (MO-951, Narishige). A new hole was opened after three to seven electrode penetrations. The search stimuli were tone bursts. Extracellular single-unit activity was discriminated using a template-matching discriminator (ASD, AlphaOmega Engineering) in 50-μs time resolution.

Daily recording sessions lasted 3–5 h over 2–6 mo. The characteristic frequency (CF) of the first isolated unit of each track was recorded and marked on a map corresponding to the coordinate of the manipulator stage. Based on the frequency reversal of the tonotopic gradient, we drew an estimated border between A1 and the posterior auditory field (PAF). This border was further confirmed by a histological procedure. Some A1 tracks near the border were re-applied with electrolytic lesions (15 μA, 10 s). The animal was deeply anesthetized and perfused with 10% formalin. The brain was removed and photographed. The cerebral cortex was cut into coronal sections and stained with neutral red. The border between A1 and PAF was adjusted according to the lesion location and the track coordinates.

Sound generation and delivery

The sound waveform was digitally generated using user-written programs under a MATLAB (Mathworks) environment. The signals were fed into a 16-bit D/A converter (PCI-6052E) at a sampling frequency of 100 kHz and to an 8-pole Chebyshev filter (P-86, NF Electric Instruments) with a high cut-off frequency of 20 kHz. The tones were played through a speaker (K1000, AKG) placed 2 cm from the auricle contralateral to the recording site. We calibrated the sound delivery system between 0.128 and 16 kHz at the frequency step of 8 Hz, and the output varied by ±5 dB. Harmonic distortion was less than –60 dB.

Pure tone stimuli and data analysis

When a single unit was isolated, we recorded and analyzed its pure tone response properties on-line to characterize the cortical region and to select the appropriate parameters of FM stimuli. First, a rough frequency response area (FRA) was measured by presenting 120 tone bursts (160-ms duration with 5-ms rise/fall time) combined with 20 frequencies (equally spaced across a 2-octave range centered at the audio-visually estimated CF) and 6 SPLs (20–70 dB in 10-dB steps). This FRA served to identify the unit’s best SPL, at which the strongest tone-evoked responses were acquired. All the following experiments were performed at the best SPL. Second, a high-resolution tuning curve was constructed by presenting 125 frequencies (500-ms duration with 5-ms rise/fall time) covering 0.128–16 kHz in a linear step of 128 Hz, or 0.128–8 kHz in 64-Hz steps.

Figure 1A shows the spike activities of an example cell responding to 125 tone bursts. The “spontaneous rate” was the average spike rate from 125 tone bursts at 500-ms prestimulus duration (–0.5 to 0 s). The “response threshold” was defined as the spike rate level of twice the SD above the spontaneous rate. Based on careful inspection of the raster plot, we flexibly selected a time window (0–50, 0–100, 0–150, or 0–500 ms) to calculate the response spike rate, covering the duration of an obvious increase in spike density. The time window for this example neuron was 0–500 ms. The “tuning curve” (Fig. 1B) was the function of the driven rate (response rate – spontaneous rate) against frequency, which was smoothed by a Gaussian function with an SD of twice the stimulus frequency interval. The best frequency (BF) was the frequency corresponding to the tuning curve maximum. The bandwidth of the tuning curve (BW) was calculated by the peak width at half-maximum height (Fig. 1B, dotted line). The frequency range corresponding to the BW (Fig. 1B, horizontal bar) was termed the tonal receptive field (TRF). For neurons with multiple-peaked tuning curves, separated by troughs lower than the half-maximum height, BW was the sum of BWs of each peak.

Next, 1-ms bin peri-stimulus time histogram (PSTH) (Fig. 1C) was constructed within the cell’s TRF (1.9–4.1 kHz in this example) and smoothed by a gaussian function with 5-ms SD. The spontaneous rate (the mean spike rate during the 500-ms prestimulus period) was subtracted from PSTH, calculating the driven rate. The threshold of PSTH was 2 SD of spike rates of the prestimulus 500 bins (Fig. 1C, dotted line). The “on response duration” of pure-tone stimuli was defined as the sum of the 1-ms bins, which was higher than the threshold. This summation was only conducted during the 0–500 ms of the stimulus period; the offset response was not included. The on response duration of the example cell was 485 ms.

Some neurons also showed a phasic response locked to the sound offset. Such a response was termed an “off response,” since it always occurred just after the sound offset. The tuning curve of the off
response (OFF tuning curve) was also calculated using the time window matching its duration.

**FM tone stimuli and data analysis**

FM tones are presented as linear FM ramps at the best SPL. We individually selected the frequency extent of the FM sweep to traverse all the cell’s responsive frequencies. The FM sweeps had an additional amplitude rise/fall time of 5 ms during which the frequency was maintained constant. For each cell, six different speeds were successively selected from 2, 4, 8, 16, 32, 64, 128, or 256 Hz/ms. One limitation of the selection of speed was to ensure that all the sweep durations were between 10 and 2,000 ms. The 10-ms limit was to make the sweep duration longer than the rise/fall duration. The 2,000-ms limit was to avoid an uncomfortable sensation for the cats.

The FM sweep at each speed was repeated 10 times in both upward and downward directions, constructing a 120-trial stimulus block (6 speeds × 2 directions × 10 repetitions). The 120 stimuli were presented in random order with an interstimulus interval >1 s.

The raster plots and PSTHs of FM responses (Fig. 2, A and B) were constructed using a method similar to the analysis of pure tone responses. The spontaneous rate and threshold of FM PSTHs were also defined similarly to those of pure-tone PSTHs; however, the “FM response duration” was computed in a time window from stimulus onset to 100 ms after stimulus offset, unlike the fixed 500-ms time window for computing the pure tone response duration to include responses lasting longer than the short sweeps (Fig. 2, A and B, 1st 2 panels).

The cell’s FM speed preference was assessed by the function of FM response duration versus sweep speed (duration-speed function) and

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Raster plots and PSTHs of a representative cell in response to 10 repetitions of upward (A, from 0.2 to 8 kHz) and downward (B, from 8 to 0.2 kHz) FM sweeps at different FM speeds. *Inset:* exact stimulus duration corresponding to each FM speed. Note that each duration contains a fixed 5-ms rise and 5-ms fall time. Each PSTH represents the mean driven rate (1-ms bin, smoothed by gaussian function with 5-ms SD) for 10 presentations of the same FM stimulus. Long vertical lines indicate stimulus onset. Horizontal bars below PSTHs indicate stimulus durations. Dotted vertical lines indicate when FM sweeps reach the edge frequencies of TRF. C: PSTHs of upward (solid line) and downward (dotted line) FM sweeps at 4 Hz/ms and tuning curve (shaded) are plotted together in the same frequency dimension.
the function of response amplitude versus sweep speed (amplitude-speed function), respectively. In the amplitude-speed function, the response amplitude was the peak amplitude of PSTH (the greatest number of spikes in a given 1-ms bin). We did not adopt some response duration-related parameters, such as the total spike count or average spike count per sweep, because the FM response duration proportionally changed with the sweep duration in sustained response cells and remained constant in phasic response cells (see RESULTS for detailed examples). These parameters are inappropriate for comparing between cells with different temporal response patterns.

RESULTS

Pure tone and FM responses of an example cell

In total, 197 cells were recorded from both hemispheres of three awake cats. One representative cell with the most simple response pattern is shown in Figs. 1 and 2. The values of computed parameters of tonal responses are presented in Table 1. For this cell, we applied FM stimuli sweeping between 0.2 and 8 kHz to cover the cell’s entire range of response frequencies (1–7 kHz, as shown in Fig. 1A). The tested FM speeds were 4, 8, 16, 32, 64, and 128 Hz/ms. The corresponding stimulus durations were displayed in the inset diagram in Fig. 2. Note that each sweep included a fixed 5-ms rise and 5-ms fall time.

Several features in Fig. 2, A and B, are noteworthy. First, at slow sweep speeds, the cell showed continuous discharges during the middle portion of the FM sweeps. The height of PSTH gradually increased to its peak and then decreased to a spontaneous level. This temporal pattern of FM response contrasts with the temporal pattern of tonal response (Fig. 1C), which showed the strongest response within the first 100 ms of tonal stimulus and gradually adapted to the later stimulus. This suggests that neuron discharge could be continuously modulated by the dynamic frequency signal of the FM sweep rather than adapting early to the stimuli. Second, the duration of the FM response changed in a systematic fashion across FM sweeps with different speeds. With an increase in the sweep speed (decrease in sweep duration), the response duration proportionally decreased. When the FM speed was ≥64 Hz/ms, the sweep duration became so short (<122 ms) that the response outlasted the stimulus duration. Third, the time of the response peak also changed in a systematic fashion. The faster the sweep speed, the earlier the response peak appeared. Fourth, the upward and downward FM sweep responses showed a rough mirror image in the time domain.

All these features may be interpreted to suggest that a particular effective range of instantaneous frequencies evokes neuronal responses. The dotted vertical lines in PSTHs mark the moments when FM sweeps reached the frequencies corresponding to the low and high half-height edges of the cell’s tonal tuning curve (1.9 and 4.1 kHz). It is apparent that stronger FM responses occurred when the FM sweep traversed the TRF, suggesting that the effective instantaneous frequency range corresponds to the TRF. We further compared the cell’s tuning of instantaneous FM frequency and tonal frequency. Because linear FM sweeps were used, the time axis in FM PSTH can be easily converted into a frequency axis to construct the instantaneous frequency tuning curve (Fig. 2C). For a FM sweep from 0.2 to 8 kHz at the speed of 4 Hz/ms, the first five bins of PSTH corresponded to the fixed start frequency of 200 Hz and the next 1,950 bins (1 ms/bin) to frequencies from 204 to 8,000 Hz in 4-Hz steps. A similar method was applied to the PSTH of the downward sweep in a reversed time/frequency order. The tonal tuning curve was also interpolated in 4-Hz steps and superimposed on PSTHs of upward and downward sweeps in Fig. 2C. The instantaneous frequency tuning fitted well with tonal tuning, suggesting that the dynamic frequencies of slow FM sweeps could be represented by the neuron’s instantaneous discharge rate, corresponding to the mean discharge rates representing steady tones. However, such an instantaneous frequency representation has two obvious limits: the FM speed could not be too fast and the bell-shaped instantaneous response profile caused uncertainty in representing two different frequencies that evoked the same response magnitude.

Population features of tonal responses

We found that the temporal response patterns of A1 cells related to BF. Figure 3A shows the scatter plot of BF versus on response duration (the response during the stimulus period). There are 11 cells on the y-axis of Fig. 3A, indicating that they had only off responses without on responses (on response duration was 0). The BF of these cells was the frequency evoked the strongest off response. One notable pattern in Fig. 3A was that cells with long-duration responses were concentrated in a low BF area, whereas those with short-duration responses were dispersed throughout the BF domain. This tendency was more apparent on the bar graph of BF distribution (Fig. 3B). Although our sample was biased toward low BF, 53.8% low BF cells (<5 kHz) had a long on response duration (>0.25 s), whereas only 20.9% high BF cells (5 kHz) had a long on response duration. The BF distribution of long-duration (>0.25 s) cells was significantly lower than that of short-duration (<0.25 s) cells (25th, 50th, and 75th percentiles: 0.5, 1.5, and 4.4 vs. 1.5, 6.7, and 10.2 kHz; Wilcoxon rank-sum test, P < 0.001). Thus cells with sustained tonal responses were more frequently found in the low BF region of A1.

The on response duration was also related to BW, as shown by the scatter plot of BW versus the on response duration (Fig. 3C). Several cells without on response were plotted on the y-axis to indicate their BWs of the off response. Cells with a long on response generally had a narrow BW, whereas those with a short response had various BWs. The similarity between the relationships of on response duration and BW and on response duration and BF is reasonable, because it is well known that BF and BW are systematically correlated in A1 cells.

### TABLE 1. Values of computed response parameters of example cells

<table>
<thead>
<tr>
<th>Figure No.</th>
<th>BF, Hz</th>
<th>BW, Hz</th>
<th>on Response Duration, ms</th>
<th>FM DSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>3,200</td>
<td>2,304</td>
<td>485</td>
<td>0.16</td>
</tr>
<tr>
<td>4</td>
<td>8,704</td>
<td>1,024</td>
<td>124</td>
<td>−0.31</td>
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<td>5</td>
<td>4,608</td>
<td>2,432</td>
<td>28</td>
<td>−0.5</td>
</tr>
<tr>
<td>6</td>
<td>8,320</td>
<td>6,784</td>
<td>70</td>
<td>−0.47</td>
</tr>
<tr>
<td>7</td>
<td>9,216*</td>
<td>8,832*</td>
<td>38*</td>
<td>0.39*</td>
</tr>
<tr>
<td>8</td>
<td>1,472</td>
<td>768</td>
<td>194</td>
<td>−0.28</td>
</tr>
<tr>
<td>9</td>
<td>14,080</td>
<td>15,744</td>
<td>73</td>
<td>−0.18</td>
</tr>
</tbody>
</table>

*Computed on the basis of off response. BF, best frequency; BW, bandwidth of the tuning curve; DSI, direction preference index.
In addition to the ON response, 120 of the 197 (60.9%) recorded cells showed OFF responses. The occurrence of OFF responses also depends on the type of the ON response pattern. Figure 3D shows the separate distribution histograms of the ON response duration in cell groups with and without OFF response. The percentage of cells with OFF responses was 26.2% in cells with an ON response >0.45 s, whereas the percentage was 86.3% in cells with an ON response <0.05 s. Thus OFF responses more frequently occurred together with a phasic ON response. This feature was more obvious when we separated the scatter plot of BW versus ON response duration (Fig. 3C) into two plots, based on the absence or presence of OFF response (Fig. 3, E and F).

Pure tone and FM responses of other example cells

More example cells corresponding to different positions in the BW versus ON response duration distribution (Fig. 3C, filled circles) are presented to show the variety of cell responses and to identify the types of responses observed in our sample. The spectral-temporal response patterns of these examples are shown in Figs. 4–10 (numbers near the filled circles in Fig. 3 represent the number of the figure displaying the example cell). Detailed values of the computed tonal response parameters are presented in Table 1.

A representative cell with a long ON response and narrow BW (Fig. 3, filled circle 1) has already been presented in Figs. 1 and 2. Figure 4 displays a cell with a shorter tonal response and narrow BW (Fig. 3, filled circle 4). For this cell, we applied FM stimuli swept from 7 to 11 kHz. Similarly to the cell in Fig. 1, the duration of the FM response also decreased proportionally to the increase of sweep speed; however, one difference was that the FM responses failed to last throughout the period when the FM sweep traversed the cell’s TRF (shown by the dotted lines on PSTHs). The comparison between PSTHs of 4-Hz/ms FM and the tonal tuning curve (Fig. 4D) indicates that instantaneous frequency tuning was narrower than tonal tuning; thus such a cell has less ability to follow a continuous stimulus because of adaptation.

Figure 5 shows a cell with a much shorter ON response. When the cell was stimulated by a slow FM sweep (2–7 kHz), such as 4 Hz/ms, a transient response was evoked when the instantaneous frequency was in the middle of TRF (Fig. 5D). The response duration was 75 and 36 ms for upward and downward FM sweeps, respectively, which was quite shorter...
than the 608-ms time interval required for the 4-Hz/ms FM traversing the TRF in 2,432 Hz width (2432/4 = 608). With the increase of sweep speed, the response duration was not obviously changed, but the response amplitude was increased, especially for downward sweeps. The phasic FM response pattern coincided with the cell’s phasic tonal response pattern, suggesting that this cell rapidly adapted to both steady and dynamic tones. A phasic cell with a relatively broad frequency tuning (Fig. 6) showed a similar FM response pattern, except for the FM direction preference.

Figure 7 shows a cell with only an OFF response. The BF, BW, and duration of the OFF response are presented in Table 1. In response to FM sweeps from 0.128 to 16 kHz, this cell exhibited multipeaked phasic responses and a preference for slow sweep speeds (Fig. 7, B and C). One of the peaks of upward FM responses occurred when the FM sweep left the TRF from the high-frequency edge (Fig. 7B, right dotted lines). Surprisingly, prominent responses occurred when the FM sweep was outside of the TRF (Fig. 7D). These responses were, however, rather reliable across different presentations of the same FM stimulus, as shown by the raster plots in Fig. 7, B and C. Thus the FM responses of a cell with OFF responses may reliably reflect the time when FM sweeps across specific frequencies, which could not be completely explained by the cell’s excitatory tonal response.

More complex examples of neurons with both ON and OFF responses are shown in Figs. 8 and 9. The cell shown in Fig. 8 had a similar ON and OFF TRF. The temporal pattern of the tonal ON response is between that of the cells shown in Figs. 1 and 4; however, the FM response pattern was different from those of the above two examples. The cell exhibited two separate responses to a slow FM sweep from 0.128 to 4 kHz (Fig. 8, B and C). With increasing sweep speed, one response peak corresponding to higher stimulus frequency (Fig. 8, B and C, dark arrows) decreased gradually. On the other hand, the response peak corresponding to lower stimulus frequency (Fig. 8, B and C, light arrows) increased in amplitude, but decreased in duration. Both response peaks were obviously outside the ON TRF (Fig. 8, B and C, dotted lines). We compared the PSTHs of 4-Hz/ms FM with the ON and OFF tuning curves in Fig. 8, D and E, respectively. The TRFs of ON and OFF responses do not account for the FM responses. It can be assumed that the later response to upward sweeps was a rebound response after the stimulus traversed the cell’s possible inhibitory receptive field between 3 and 4 kHz (Fig. 8A), although this rebound facilitation hypothesis could not explain why the cell responded to the downward FM when it had just entered the inhibitory field. Thus the presence of an OFF response complicated the cell’s FM response pattern.

Figure 9 shows a cell with a phasic ON response to all the frequencies in the range of our sound delivery system (0.128–16 kHz) and OFF responses to most of the frequencies (3–16 kHz). For such a broad tuning cell, we presented FM stimuli sweeping between 0.128 and 16 kHz. This cell showed a phasic onset response to both upward and downward sweeps, which was understandable because the FM sweeps started within the cell’s ON TRF. The cell also responded to the later portion of slow sweeps with a multipeaked response pattern.
FIG. 5. Tonal and FM response properties of an example cell with a phasic ON response. A: raster plots for 125 tonal stimuli. B and C: PSTHs of upward and downward FM sweeps (sweep range: 2–7 kHz). Dotted vertical lines indicate the edge frequencies of TRF, measured by the 0- to 50-ms time window. D: PSTHs of FM sweeps at 4 Hz/ms are superimposed on the cell’s tuning curve. Refer to Figs. 1 and 2 for other captions.

FIG. 6. Tonal and FM response properties of the other example cell with a phasic ON response. A: raster plots for 125 tonal stimuli. B and C: PSTHs of upward and downward FM sweeps (sweep range: 0.1–16 kHz). D: PSTHs of FM sweeps at 8 Hz/ms are superimposed on the cell’s tuning curve. Refer to Figs. 1 and 2 for other captions.
which did not clearly relate to the cell’s OFF tuning curve (Fig. 9D).

As shown by individual examples (Figs. 7–9), unpredictable FM responses were more frequently observed in cells with OFF response to pure tones. Compared with only 8% (6/77) of the cells without tonal OFF response, 80% (96/120) of the cells with OFF responses exhibited one or more FM response peaks that cannot be explained by the cell’s tonal responses. Thus the production of unpredictable FM responses may share some similarities with that of tonal OFF responses. The second feature of unpredictable FM responses is the slow speed preference; the slower the sweep speed, the more frequent and higher the response peaks (Figs. 7–9).

**Population features of FM responses**

The above examples show the presence of multiple response patterns in A1 cells. Although the response patterns continuously changed among the cell population (Fig. 3), to summarize the response features of different cells, we grouped the 197 cells into several subgroups according to their tonal response patterns.

First, 53 cells located in the bottom right quarter of the distribution in Fig. 3E were grouped as sustained ON cells. They showed sustained (ON response duration > 250 ms), narrowly tuned (BW < 8 kHz) tonal responses, and no OFF response (like the cell in Fig. 1). The duration of their FM response proportionally decreased with the increase of sweep speed, as shown by the mean function of response duration against sweep speed (duration-speed function; Fig. 10A). The response duration of 32-Hz/ms FM sweep was positively correlated with the BW of TRF (Fig. 10B; \( r = 0.43, P < 0.003 \)), suggesting that the FM response duration corresponds to the duration of the sweep traversing the cell’s TRF. The mean amplitude-speed function (peak driven rate vs. sweep speed) of the 53 sustained ON cells showed a low-pass type (Fig. 10C). Both the duration-speed and amplitude-speed functions suggest a slow speed preference of sustained ON cells.

Second, 17 cells located in the bottom left quarter of the distribution in Fig. 3E (ON response duration < 250 ms and BW < 8 kHz) were grouped as phasic ON cells. They showed a phasic and narrowly tuned tonal response and no OFF response (like the cell in Fig. 5). The FM response duration of phasic ON cells was less affected by the sweep speed (Fig. 10D) and was uncorrelated to the BW (Fig. 10E). The amplitude-speed function was a high-pass type (Fig. 10F). Third, 11 cells with only OFF response (like the cell in Fig. 7) were grouped as OFF cells, which are shown on the ordinate of the scatter plot of ON response duration versus BW (Fig. 3F). Both their duration-speed and amplitude-speed functions showed a low-pass type (Fig. 10, G and I). The FM-response duration was positively correlated with the BW of the OFF tuning curve (Fig. 10H; \( r = 0.61, P = 0.046 \)).

The fourth subgroup is the 70 cells with narrowly tuned ON and OFF responses (like the cell in Fig. 8), termed ON-OFF cells.
They are located in the bottom half of Fig. 3F, indicating a narrow TRF of ON response (BW < 8 kHz). They exhibited a complex FM response pattern combining the features of phasic ON cells and OFF cells (Fig. 8), resulting in a low-pass-type duration-speed function (Fig. 10J) and all-pass-type amplitude-speed function (Fig. 10M). The FM response duration correlated to neither the BW of ON response (Fig. 10L; \( r = 0.04, P = 0.77 \)) nor to that of OFF response (data not shown; \( r = -0.07, P = 0.59 \)).

Finally, the remaining 46 cells with a BW of ON response >8 kHz were termed wide-tuning cells. These cells are located in the top halves of Fig. 3, E and F. For all wide-tuning cells, we applied FM stimuli sweeping between 0.128 and 16 kHz. As shown in the example in Fig. 9, the total duration of multipeaked responses decreased with the increase of sweep speed (Fig. 10N) but correlated to neither the BW of ON response (Fig. 10O; \( r = 0.14, P = 0.36 \)) nor to that of OFF response (data not shown, \( r = 0.11, P = 0.49 \)). The maximum response amplitude was independent of sweep speed (Fig. 10P).

**Sweep direction preference**

As shown in Figs. 4, 5, and 7, some cells show an obvious sweep direction preference. We calculated the direction preference index (DSI) by the following equation: \( \text{DSI} = (RU - RD)/(RU + RD) \). RU and RD represent the mean driven rates (time window is from stimulus onset to 100 ms after stimulus offset) of upward and downward sweeps, respectively. DSI can vary between 1 and –1, where positive and negative signs indicate a preference for upward and downward sweeps, respectively. DSI may vary with the sweep speed in the same cell. We selected the DSI with the maximum absolute value to evaluate the cell’s direction preference. The DSI values of example cells are presented in Table 1. The DSI of each cell was plotted against BF in Fig. 11A, in which the BF of cells without ON response was decided by referring to the cell’s OFF response. DSI is negatively correlated with BF (\( r = -0.34, P < 0.01 \)), suggesting that cells with a high BF tended to have a negative DSI, whereas those with a low BF tended to have a positive DSI. This tendency was more apparent when we plotted the mean and SD of DSI among cells located in...
different BF bands (Fig. 11B). Such a correlation between DSI and BF was also observed in all subgroups of our tested cells (sustained ON cells, \( r = -0.29, P = 0.04 \); phasic ON cells, \( r = -0.5, P = 0.03 \); OFF cells, \( r = -0.69, P = 0.02 \); ON-OFF cells, \( r = -0.39, P = 0.001 \); wide-tuning cells, \( r = -0.37, P = 0.01 \)). Thus A1 cells in the low-BF area prefer an upward sweep direction, whereas those in the high-BF area prefer a downward direction.

**DISCUSSION**

A major finding of this study is that A1 cells in awake cats responded to tonal and FM stimuli in various temporal response patterns, which were rarely reported in previous anesthetized studies. The potential functions of the multiple response patterns and the comparison with previous reports are discussed in the following sections.

**Diverse tonal response patterns**

Generally, the tonal responses of our A1 cells could be decomposed into three basic patterns: sustained ON, phasic ON, and OFF responses. Individual cells may exhibit only one of the basic response patterns or a combination of two or three. Moreover, the boundary between sustained ON and phasic ON responses is obscure (Fig. 3A). Some cells have an intermediate ON response (Fig. 4). All these factors contribute to the heterogeneity of tonal responses in A1.

One noticeable feature is that the sustained ON response was mostly observed in low-BF cells, whereas the phasic ON response was observed equally in both low- and high-BF cells (Fig. 3, A and B). Consequently, sustained responders usually showed a narrow BW, whereas phasic responders showed various BWs (Fig. 3C). This trade-off in on response duration and bandwidth may suggest a functional differentiation among A1 cells; that is, sustained cells may be specialized in encoding spectral information, suggested by their capability to represent a selective range of frequencies and continuously track them. On the other hand, phasic cells, especially those with a broad BW, may serve to encode temporal information, since they can precisely represent the sound onset and offset irrespective of the frequency.

**Diverse FM responses associated with tonal responses**

Different tonal response patterns associate with different FM responses. A cell with a sustained ON tonal response shows a sustained FM response, as a FM sweep traverses the cell’s receptive field (Fig. 2). Similarly, a cell with a phasic ON tonal response also shows a phasic FM response (Fig. 5). The OFF response of tonal stimuli associates with a multipeaked type of FM response (Fig. 7), in which the response peaks are mostly unpredictable from tonal responses. Cells with both ON and OFF responses to pure tones usually exhibit a complex FM response pattern combining the features of phasic ON cells and OFF cells (Fig. 8). Cells with broad frequency tuning (Fig. 9) show a dominant onset response to all kinds of FM sweeps corresponding to their phasic ON tonal response component and several unpredictable later responses that may associate with their OFF response to pure tones. Thus their major role may be
to detect the onset signal of sounds rather than encoding detailed FM signals. The presence of diverse tonal and FM response patterns suggests that A1 cells of awake cats may respond to all kinds of natural complex sounds in multiple discharge patterns.

**Comprehensive representation of FM parameters**

The functional consequence of the diversity of FM response patterns may be to broaden the range of FM parameters represented by A1. In the natural environment, cats will hear
many complex sounds with various FM parameters, such as frequency extent, sweep speed, and direction. The richness of response patterns ensures that most FM parameters could be sufficiently represented by A1 cells.

The frequency extent of an FM sweep is read on the basis of the cell’s receptive field. A cell’s receptive field of FM stimuli usually matches well with that of tonal stimuli, such as sustained ON cells, but there are also some unmatched cases, such as cells with OFF responses. A specific FM sweep will selectively elicit a group of cells whose receptive fields are traversed. Thus the FM extent is represented by a sequence of spatially distributed neuronal responses. Additionally, the instantaneous FM frequency of a slow sweep might be also encoded by the instantaneous discharge rate of sustained responders (Fig. 2C); however, the frequency resolution of such encoding is highly dependent on the sweep speed, which limits its application to fast FM sweeps. Because many animal vocalizations, such as a cat’s meow (Tian and Rauschecker 1998) or bird chirp (Bar-Yosef et al. 2002), contain a slow FM component (<80 Hz/ms), the instantaneous tuning method may be useful to process these communication sounds.

The sweep speed can be represented by various cells in multiple ways, including the low-pass-type response duration function and response amplitude function of sustained ON cells and OFF cells and the high-pass-type response amplitude function of phasic ON cells (Fig. 10). The sweep direction can be represented by the mean firing rate of all kinds of cells (Fig. 11). Moreover, PSTHs of upward and downward sweeps are different in most cells (Figs. 4–9), suggesting that sweep direction is also distinguishable according to direction-specific temporal patterns.

With the use of these multiple encoding strategies, FM signals of a complex sound can be decomposed and represented by a population of A1 cells. The output of the A1 process may be further represented by higher stages of auditory hierarchy, such as the secondary auditory cortex, to detect specific combinations of vocalization elements (Schreiner and Cynader 1984).

Comparison with previous studies on FM response patterns

In anesthetized cats, high-resolution mapping showed the systemic spatial distribution of FM speed preference along the dorsoventral axis of A1 (Mendelson et al. 1993). Generally, neurons in the most dorsal or ventral part of A1 preferred fast speeds, whereas those in the central preferred slow speeds. Because it is difficult to sample the cortex of awake animals evenly and extensively to obtain a high-resolution map, we did not relate the response heterogeneity to subregional topographies within A1. In the awake experiment, recording sites are only checked after completing the last recording. There remains a possibility that a few PAF cells were also collected in our A1 sample, although histological measurements (see methods for details) were taken to avoid this.

On the other hand, this study on awake cats showed the heterogeneity of neuronal response patterns, which were not observed under anesthesia. A directly comparable report was the study of Heil et al. (1992b) on the A1 of anesthetized cats. Although they also used linear FM sweeps traversing the cell’s receptive field at speeds ranging from 2 to 2,048 Hz/ms, covering the speed range used in this study (2–256 Hz/ms), no variety of the temporal response patterns was mentioned in their report; thus anesthesia may reduce the range of response heterogeneity.

In the awake state, a pioneering study (Whitfield and Evans 1965) reported four types of FM responses in cat A1. 1) Cells started firing as an FM tone crossed the boundary into the cell’s TRF and continued to fire until it left. These cells obviously correspond to sustained ON cells in this study. 2) Cells started and stopped firing when FM tones were wholly within TRF. These cells may correspond to our phasic ON cells. 3) Cells were fired when FM tones were outside TRF. These cells may correspond to cells with OFF response to tonal stimuli in this study. 4) Cells responded to FM tones but not to steady tones. Because we used pure tones as searching stimuli, we did not include such cells in this report.

The other recent study on the A1 of awake monkeys (Atencio et al. 2007) also reported that sustained FM responses were more often found in the awake experiment than under anesthesia. Although they did not compare FM responses with the responses evoked by steady tones, inspection of their example figures showed that the FM response features of the example cell in their Fig. 2 are similar to those of our sustained ON cells (our Fig. 2); the example in their Fig. 3 is similar to our phasic ON cells (our Figs. 5 and 6), and the example in their Fig. 4 may be between the former two examples, corresponding to our intermediate cell (our Fig. 4). Thus multiple FM response patterns may also exist in the A1 of awake monkeys.

Atencio et al. showed that FM response parameters could be predicted by tone-evoked receptive field parameters, but some significant deviations also exist between predicted and actual response parameters. Our results indicate that the predictability of FM responses depends on the pattern of associated tonal response. FM responses of cells with sustained tonal responses are relatively easy to predict (Fig. 2D). The most unpredictable FM responses are observed in cells with OFF responses to pure tones (Figs. 7–9). One possible mechanism is that such an FM response was simply a rebound response occurring after the sweep traverses the cell’s inhibitory receptive field, where spontaneous activities were suppressed by tonal stimuli. Be-
cause there is no reliable reference to precisely define the amplitude of inhibitory effect, especially for cells with a low spontaneous rate, we could not examine this issue in-depth using our extracellular recording data. Recently, in vivo whole cell recordings (Liu et al. 2007; Xie et al. 2007; Zhang et al. 2003) showed that auditory neurons often receive spectrally broader and more complex inputs than suggested by the receptive field observed from extracellular recordings. Inputs generated by FM sweeps are likely to interact in ways that are invisible with extracellular recordings. Thus as noted in previous studies (Atencio et al. 2007; Barbour and Wang 2003; Bar-Yosef et al. 2002; Escabi and Schreiner 2002), models based on a linear summation of energy within a spectral-temporal receptive field are insufficient to predict FM responses.

Previous reports on FM direction preference

Previous experiments have shown that A1 neurons are sensitive to FM sweep direction (Heil et al. 1992b; Kowalski et al. 1995; Mendelson and Grasse 1992; Nelken and Versnel 2000; Phillips et al. 1985), and the direction preference is topographically ordered in parallel with BF or CF (Godey et al. 2005; Heil et al. 1992a; Mendelson and Ricketts 2001; Zhang et al. 2003). For example, A1 neurons with a higher CF (>14 kHz) tended to prefer downward sweeps, whereas those with a lower CF (<8 kHz) tended to prefer upward sweeps in rats (Zhang et al. 2003); however, a recent study on awake owl monkeys did not show a significant correlation between direction preference and CF (Atencio et al. 2007), possibly because of incomplete sampling of neurons using an implanted array of microelectrodes. A significant negative correlation between DSI and BF was also found in these data of awake cats (Fig. 11), confirming that this tendency commonly exists across species.

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