

# Spectral Integration in A1 of Awake Primates: Neurons With Single- and Multi-peaked Tuning Characteristics

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Submitted 5 April 2001; accepted in final form 4 October 2002

**Kadia, Siddhartha and Xiaoqin Wang.** Spectral integration in A1 of awake primates: neurons with single- and multi-peaked tuning characteristics. *J Neurophysiol* 89: 1603–1622, 2003; 10.1152/jn.00271.2001. We investigated modulations by stimulus components placed outside of the classical receptive field in the primary auditory cortex (A1) of awake marmosets. Two classes of neurons were identified using single tone stimuli: neurons with single-peaked frequency tuning characteristics (147/185, 80%) and neurons with multi-peaked frequency tuning characteristics (38/185, 20%), referred to as single- and multi-peaked units, respectively. Each class of neurons was further studied using two-tone paradigms in which the frequency, intensity, and timing of the second tone were systematically varied while a unit was driven by the first tone placed at a unit's characteristic frequency (CF) if it was single-peaked or at one of multiple spectral peaks if it was multi-peaked. The main findings were: 1) excitatory spectral peaks in the frequency tuning of the multi-peaked units were often harmonically related. 2) Multi-peaked units showed facilitation in their responses to combinations of two harmonically related tones placed at the spectral peaks of their frequency tuning. The two-tone facilitation was strongest for the simultaneously presented tones. 3) In 76 of 113 single-peaked units studied using the two-tone paradigm, facilitatory and/or inhibitory modulations by distant off-CF tones were observed. This distant inhibition differed from flanking (or side-band) inhibitions near CF. 4) In single-peaked units, the distant off-CF inhibitions were dominated by tones at frequencies that were harmonically related to the CF of a unit, whereas the facilitation by off-CF tones was observed for a wide range of frequencies. And 5) in both single- and multi-peaked units, sound levels of two interacting tones determined whether the two tones produced excitation or inhibition. The largest facilitation was achieved by using two tones at their corresponding preferred sound levels. Together, these findings suggest that extracting or rejecting harmonically related components embedded in complex sounds may represent fundamental signal processing properties in different classes of A1 neurons.

## INTRODUCTION

A fundamental concept in neural processing of sensory information is the notion of "receptive field." The receptive field of an auditory cortical neuron (also referred to as the *frequency response area* in the literature) is defined on a two-dimensional space of the frequency and intensity (e.g., Shamma et al. 1993; Suga 1965a,b). For neurons with single-peaked tuning characteristics, the receptive field derived by single-tone stimulation is typically characterized by an excita-

tory response area with a threshold (the lowest sound level necessary to evoke a neuronal response), a characteristic frequency (CF, the frequency corresponding to the threshold), and a bandwidth (the range of frequencies surrounding the CF to which the neuron responds, usually measured at 10 dB above threshold). For neurons that display nonmonotonic rate-level functions (Pfungst and O'Connor 1981; Phillips et al. 1985; Wang et al. 1999), a best or preferred sound level is also used in the characterization. Finally, side-band inhibition that flanks the excitatory response area could be revealed by single-tone (if there is insufficient spontaneous activity) or two-tone paradigms (Shamma et al. 1993; Suga 1965a,b). The receptive field defined by these parameters is referred to here as the *classical receptive field*.

A fundamental limitation in receptive field characterization is that the nature of a receptive field is always limited or biased by the form of acoustic stimuli used to define it. In the visual cortex, a receptive field defined by spots of light or moving bars does not necessarily reflect response properties of a neuron that integrates inputs from a larger visual space. Often, such properties can only be revealed by complex visual stimuli. Indeed, there has been growing evidence that stimulus elements outside the classical visual receptive field modulate a neuron's responses to both simple and complex visual stimuli (Allman et al. 1985; Gilbert and Wiesel 1990; Kapadia et al. 1999; Knierim and Van Essen 1992; Nelson and Frost 1985; Orban et al. 1987; Tanaka et al. 1986). Accordingly, one may expect that the classical receptive field of an auditory cortical neuron cannot adequately characterize response properties when a broad-band stimulus is encountered (Nelken et al. 1994; Schulze and Langner 1999; Suga and Tsuzuki 1985).

In addition to neurons with single-peaked tuning characteristics, neurons with multi-peaked tuning characteristics have been found in the auditory cortex of several species, including bats (Suga 1994), cats (Abeles and Goldstein 1970, 1972; de Ribaupierre et al. 1972; Oonishi and Katsuki 1965; Phillips and Irvine 1981; Sutter and Schreiner 1991) and primates (Aitkin and Park 1993). While the multi-peaked tuning characteristics indicate spectral integration of inputs outside the classical receptive field, the extensive horizontal connections in the primary auditory cortex (A1) (Kadia et al. 1999; Matsubara and Phillips 1988; Ojima et al. 1991; Reale et al. 1983; Wallace

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et al. 1991; Winer 1992) suggest that integration of a broad range of spectral inputs may also take place in neurons with single-peaked tuning characteristics. In the mammalian A1, integration of multiple spectral inputs has been explored extensively in bats (Suga 1994) and to a lesser extent in other mammals (Brosch and Schreiner 1997, 2000; Brosch et al. 1998, 1999; Calford and Semple 1995; Oonishi and Katsuki 1965; Phillips et al. 1985; Schulze and Langner 1999; Shamma et al. 1993; Sutter et al. 1999). In the present study, we used a two-tone paradigm to determine the extent of inputs to an A1 neuron from outside of the classical receptive field. The assumption is that if a neuron receives inputs from more than one frequency region, it may respond to multiple spectral components in a manner not predictable from single-tone responses. The two-tone paradigm has been used in many studies (Brosch and Schreiner 1997; Brosch et al. 1999; Calford and Semple 1995; Shamma et al. 1993; Suga 1965a,b; Sutter et al. 1999) but has not been systematically tested in awake primates.

In some mammalian species such as bats, a class of specialized neurons was found in the auditory cortex (Suga et al. 1978, 1983) and subcortical nuclei (Leroy and Wenstrup 2000; Olsen and Suga 1991; Wenstrup 1999) that responds optimally to specific combinations of spectral components in sonar signals that are harmonically related. These neurons have been referred to as "combination-sensitive" neurons. In a broader sense, the combination sensitivity represents a special case of contextual sensitivity. It has been suggested that combination-sensitive neurons may account for other encoding problems encountered by the auditory system including nonsonar signals such as speech (Suga 1994). However, evidence for such combination-sensitive neurons in the auditory cortex of primates has been lacking (Schwarz and Tomlinson 1990). An important lesson from the studies of bat's auditory cortex is that well-chosen laboratory-generated stimuli can facilitate our understanding of complex sounds such as species-specific communication calls.

Many natural and man-made sounds (e.g., species-specific animal vocalizations, human speech, and music) contain harmonically related spectral components. Because the peripheral auditory system functions to segregate sounds into narrow frequency channels (von Békésy 1960), a fundamental task of central auditory systems is to re-assemble these different channels into a coherent representation for perception. How such a task is accomplished remains largely unknown. It has been known from psychophysical studies that the perception of an individual acoustic event depends on the spectrotemporal stimulus context. In a natural acoustic scene, multiple frequency components often co-exist and create spectral shapes with both coarse and fine features. The perception of a single sound is profoundly influenced by preceding, following, or concurrent sounds (Plack and Viemeister 1992; Wright 1994; Wright and Dai 1994). For example, preceding or succeeding sounds can result in elevated thresholds (Lüscher and Zwislocki 1947) or alteration of subjective pitch and loudness (Stevens and Davis 1938) of a single acoustic stimulus. Successive acoustic events may be perceived as a single stream or segregated into different streams depending on spectral composition, length, and temporal separation of the events (Bregman 1990). These perceptual processes are possibly correlated with modulatory influences on neuronal responses by stimulus components from "outside" of the classical receptive field. Characterizing these

influences and their organizations are critical for bridging neurophysiology and perception.

We used a small, highly vocal New World monkey, the common marmoset (*Callithrix jacchus*) as our animal model for the following reasons. Marmosets are highly vocal and have a well-described repertoire of communication calls (Agamaite and Wang 1997; Epplé 1968; Wang 2000; Wang et al. 1995). Marmoset vocalizations are composed of distinct spectral components that are harmonically related. Such harmonic structure is fundamental to the communication sounds of many animal species. A large portion of the auditory cortex of marmosets is located on the lateral surface of the temporal lobe, easily accessible for electrophysiological studies (Aitkin et al. 1986, 1988). We conducted these experiments under the awake condition to avoid potential complications imposed by anesthesia on cortical responses.

## METHODS

### Acoustic stimuli

Experimental stimuli included pure tones and two-tone combinations. All acoustic stimuli were delivered in free-field conditions by a speaker located ~1 meter in front of the animal. All recording sessions were conducted within a double-walled, soundproof chamber (Industrial Acoustics). The interior of the chamber was covered by 3-in acoustic absorption foam (Sonex, Illbruck). The speaker (B and W Mode DM601) had a flat ( $\pm 6$  dB) frequency response from 50 Hz to 30 kHz. Stimuli were generated digitally on a computer using the full range of a 16-bit D/A converter (DA3-4, Tucker-Davis Technologies) at a 100-kHz sampling rate and attenuated to the desired sound pressure level (PA4, Tucker-Davis Technologies). Stimuli from each set were presented in random order. Typical tone duration was 100 ms (in some cases 50-ms tones were used) with a rise-fall time of 10 ms. Inter-stimulus intervals were  $\geq 1$  s. In the two-tone paradigms, the two tones were generated separately through two channels of the D/A converter and mixed before being sent to the speaker. We tested our sound delivery system thoroughly and confirmed that harmonic artifacts were at least 43 dB lower than the fundamental at 80 dB SPL (the highest sound level used in this study). The difference grew as the intensity of the fundamental decreased.

### Preparation and recording procedure

Findings presented here were based on single-unit responses recorded from A1 of two awake marmosets ( $n = 185$ ). We have developed a chronic recording preparation in this small primate species (Lu et al. 2001). Briefly, marmosets were first adapted to sit quietly in a specially designed primate chair. Two stainless steel head-posts were then implanted so that the animal's head was immobilized during recording sessions. The skull over the auditory cortex was exposed and covered with a thin layer of dental cement and surrounded by a chamber that was sealed between recording sessions. The auditory cortex was accessed using a single tungsten microelectrode (A-M Systems, impedance 3–5 M $\Omega$  at 1 kHz) via a small hole (~1 mm) through the skull, the only opening on the skull at any given time during the recording sessions. Each recording hole was sealed by dental cement after several days of recordings. Daily recording sessions, each lasting 3–5 h, were carried out for several months in each animal. On average, two to four well-isolated single units were recorded in each session. Spike waveforms were filtered, digitized, and detected using a template-matching discriminator (MSD, Alpha-Omega Engineering) that is capable of sorting at least three single units. We typically sorted one single unit, less often two single units, from the signals recorded from a single electrode. The advantage of this discriminator was that it allowed us to continuously monitor the

quality of recorded spikes in real time while recordings were in progress and its template-matching rejected artifacts in the signals produced by animal's movements.

In our experiments, the auditory cortex was approached laterally with electrodes entering perpendicularly. Spatial location of each penetration as well as the contact to the dura surface by the tip of an electrode was visually confirmed through an operating microscope at high magnification (typically  $\times 40$ ). Single units were encountered at all cortical layers, but the majority of the recorded data were from layers 2–4, judging by the depth and response characteristics. The location of A1 was determined by three criteria: tonotopic organization of A1, reversals in the gradient of CF at borders between A1 and the rostral and caudal fields, and change of response properties at the border between A1 and the lateral field (which was more responsive to noises). Neurons in A1 generally responded well to pure tone stimuli.

### Data acquisition

Once a unit was isolated and its basic response properties were determined using single tones (e.g., CF, latency and rate-level characteristics), experimental protocols using two-tone stimuli were executed.

**PROTOCOLS FOR DETERMINING CF AND RATE-LEVEL CHARACTERISTICS.** For each isolated single unit, its CF and threshold to CF tones were first estimated manually and then determined precisely by a computer-controlled procedure. Tones at a sound level 10 dB above threshold were delivered over a range of frequencies spanning several octaves at 20 steps/octave. Each frequency was tested at least five times. This resulted in an accurate determination of the CF for the unit, calculated as the frequency that evoked the highest average discharge rate. This procedure was often repeated at several sound levels to obtain the frequency response area of the unit. Next, we measured a rate-level function (average discharge rate versus sound level) in each unit using the CF tone played at different sound levels. The threshold (Th) of the response was determined from the rate-level function. A preferred sound level (PL) was also calculated if the rate-level function was nonmonotonic.

**PROTOCOLS FOR TWO-TONE STIMULI.** Two-tone interactions were studied by varying frequency, sound level, and the onset delay between the two tones. The first tone (S1) was fixed and the second tone (S2) was varied in frequency or sound level. In most units, S1 and S2 were presented simultaneously. For a subset of units studied, the onset delay between S1 and S2 was varied. The S1 frequency was usually fixed at the CF of units with single-peaked tuning characteristics (Fig. 1) and at one of excitatory frequency peaks of units with multi-peaked tuning characteristics (Fig. 2). The frequency of the S2-tone was varied in small, linear steps over a large range of frequencies ( $\geq 2$ –3 octaves around the S1 frequency). The step size was calculated by the formula  $CF/n$  ( $n = 12$  or  $24$ ). The intensity of the S1 tone was generally kept at the preferred sound level for nonmonotonic rate-level functions or 30–40 dB above the threshold for monotonic rate-level functions. If the parameters used in the preceding protocol did not result in any marked response modulation (facilitation or inhibition) in two-tone responses as compared with single tone responses, sound levels of the S1 tone and/or S2 tone were varied and the two-tone protocol was repeated. Once we identified the S2 frequency(ies) that elicited response modulation, we then systematically tested these S2 frequencies across sound levels and determined the best S2 sound level that produced the largest response modulation. For all two-tone protocols, we tested each combination of S1 and S2 for  $\geq 10$  (usually 20) repetitions. In addition, responses to the S1 tone played alone were recorded as a control. Stimuli in each protocol were delivered in random order. However, it was not always possible to

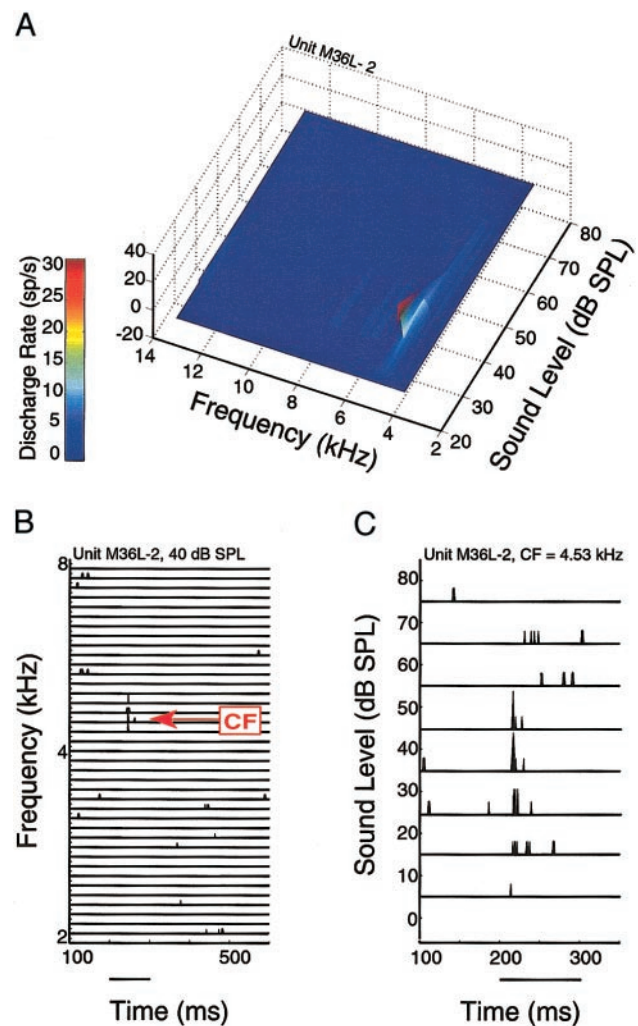


FIG. 1. An example of a single unit with the single-peaked tuning characteristics in response to single-tone stimulation. *A*: a 3-dimensional frequency response area (3-D FRA) reconstructed from responses to tones at different frequencies (2–8 kHz, 40 logarithmic steps) and sound levels (30–80 dB SPL, 5 steps). The discharge rate (vertical axis) was calculated at each sound level and frequency tested. This unit had a narrow FRA in frequency (4.3–4.7 kHz) and sound level (30–50 dB SPL). *B*: single tone responses at 40 dB SPL for the unit shown in *A*. Peristimulus time histograms (PSTHs) are shown for responses to tones ranging from 2 to 8 kHz in 40 logarithmic steps. Stimulus duration was 100 ms (onset at 200 ms) as indicated by a bar below the *x* axis. This unit has a characteristic frequency (CF) of 4.53 kHz (indicated by an arrow). *C*: PSTH of responses to the CF tone delivered across sound level (0–80 dB SPL) for the unit shown in *A* and *B*. This unit had a nonmonotonic rate-level function with a best level of 40 dB SPL and a threshold of 20 dB SPL.

evaluate the full range of parameters of two-tone stimuli in each unit due to time constraints.

### Data analysis

Single units were classified into two basic categories based on their single-tone responses: units with single-peaked tuning characteristics (referred to as single-peaked units hereafter) and units with multi-peaked tuning characteristics (referred to as multi-peaked units hereafter). Single-peaked units were responsive to single-tone stimulation in only one region of frequencies. Units that responded to more than one discrete region of frequencies were classified as multi-peaked units. A multi-peaked unit is distinct from a broadly tuned unit in that it has a region of nonresponsive frequencies separating the areas responsive to single-tone presentation.



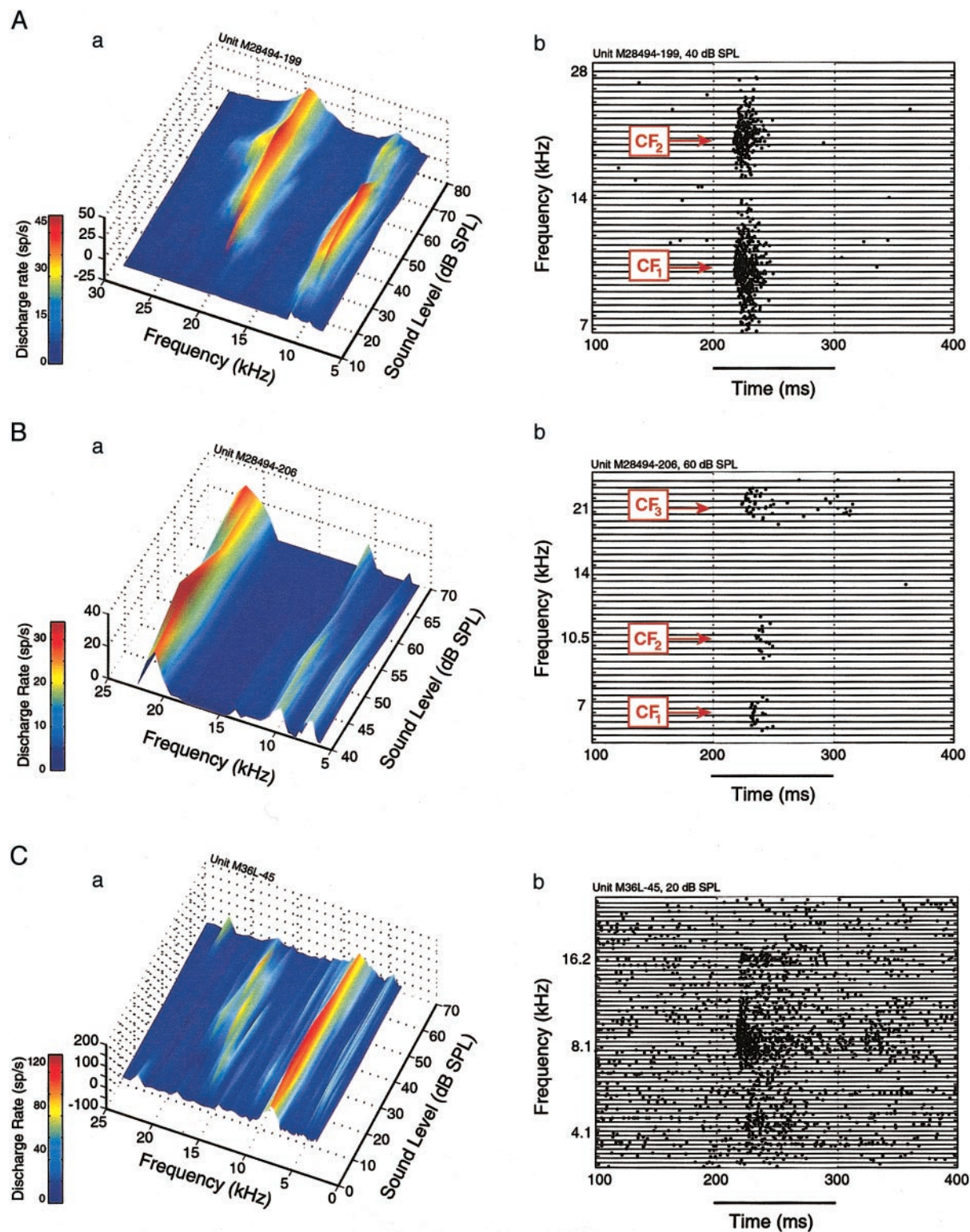


FIG. 2. Three examples of single units with the multipeaked peaked tuning characteristics in response to single-tone stimulation. A, a: 3-D FRA of a multipeaked unit. b: dot raster showing single tone responses at 40 dB SPL, a cross-section of the 3-D FRA shown in a. Stimulus duration was 100 ms (onset at 200 ms) as indicated by a bar below the x axis. This unit was responsive to 2 frequency regions. The most responsive frequency (the frequency evoking the highest discharge rate) in each region was designated as an excitatory frequency peak. This unit had 2 frequency peaks near 9.5 kHz ( $CF_1$ ) and 19 kHz ( $CF_2$ ), respectively (indicated by arrows). Note that  $CF_1$  and  $CF_2$  are harmonically related. B, a: 3-D FRA of a multipeaked unit with 3 frequency peaks. b: dot raster showing that this unit was responsive to 3 frequency regions near 7, 10.5, and 21 kHz, respectively, at 60 dB SPL. C, a: 3-D FRA of a multipeaked unit that was responsive to 3 frequency regions near 4, 8, and 16 kHz, respectively, at 20 dB SPL. b: dot raster. Note the sustained discharges near the frequency peaks.

The following measure was used to quantify the difference between single-tone and two-tone responses in both single-peaked and multi-peaked units

$$\text{Percent change in discharge rate} = 100 * (R_{S1+S2} - R_{S1})/R_{S1}$$

where,  $R_{S1+S2}$  is the average discharge rate to the S1 and S2 tones played together and  $R_{S1}$  is the average discharge rate to the S1 tone alone. Discharge rates were calculated for the time window in which strongest response modulations were observed. Spontaneous discharge rates (averaged from 200 ms preceding stimulus onset) were subtracted from  $R_{S1}$  and  $R_{S1+S2}$ . The *percent change in discharge rate* measure was defined to reflect both facilitated and inhibited two-tone responses. Two-tone responses were considered facilitated if the *percent change in discharge rate* was  $>30\%$ , or inhibited if it was less than  $-30\%$ .

In single-peaked units, two other measures, *best facilitatory peak frequency* and *best inhibitory peak frequency*, were used to quantify two-tone response modulations at particular S2 frequencies. Responses of A1 units to two-tone stimuli typically showed side-band inhibition at some S2 frequencies flanking the CF of a unit (as revealed by a reduction in discharge rates). These inhibitory regions are referred to as the *flanking inhibitory regions*. In some units, the inhibition could also be induced by S2 tones placed at frequencies outside the classical receptive field (i.e., CF-centered excitatory region plus flanking inhibitory regions). We referred to such inhibition as the *distant inhibition*. There was always a range of frequencies between the flanking inhibitory regions and distant inhibitory regions where S2 tones did not produce significant inhibition. The S2 frequency that caused the strongest distant inhibition was defined as the *best inhibitory peak frequency*. In some units, facilitation by the S2 tone could also be detected at frequencies outside the classical receptive field and is referred to as the *distant facilitation*. The S2 frequency that caused the strongest distant facilitation was defined as the *best excitatory peak frequency*. There could be more than one S2 frequency that caused facilitated or inhibited two-tone responses in a unit. For each unit, in addition to the best facilitatory (inhibitory) peak, there may be other secondary facilitatory (inhibitory) peaks where two-tone responses produced  $>30\%$  increase (decrease) in discharge rate. We also calculated S2 frequencies corresponding to these additional facilitatory (inhibitory) peaks.

## RESULTS

The results described in this report were from experiments performed in two awake marmoset monkeys. A total of 185 single units were recorded and classified into two categories based on their single-tone responses: units with single-peaked tuning characteristics ( $n = 147$ , 80% of total) and units with multi-peaked tuning characteristics ( $n = 38$ , 20% of total).

### *Existence of neurons with multi-peaked tuning characteristics*

A1 neurons typically have one CF-centered excitatory response area, as shown by an example in Fig. 1. We observed in our experiments a substantial number of A1 units that had multiple, discrete excitatory response areas as revealed by single tones. Figure 2 shows three examples of such units, referred to as the multi-peaked units. Each excitatory region can be described by the same parameters used to describe units with single-peaked tuning characteristics (i.e., CF, threshold, rate-level characteristics). In this report, we refer to the CF of the peak with the lowest frequency as  $CF_1$  in a multi-peaked unit. The CFs of the additional peaks are referred to as  $CF_n$  ( $n = 2, 3, \dots$ ). A three-dimensional (3-D) response area for a multi-peaked unit is depicted in Fig. 2Aa, where the discharge

rate is plotted against both frequency and sound level. This unit responded strongly to tones of frequencies near 9.5 kHz ( $CF_1$ ) and 19 kHz ( $CF_2$ ) at 40 dB SPL (Fig. 2Ab, a cross-section of the 3-D frequency response area shown in Fig. 2Aa). Furthermore, this unit had nonmonotonic rate-level functions at both  $CF_1$  and  $CF_2$  (Fig. 2Aa) but with different preferred sound level (50 dB SPL at  $CF_1$ , 60 dB SPL at  $CF_2$ ). Note that multi-peaked units have regions of nonresponsive frequencies separating the excitatory regions across all sound levels. An important feature of multi-peaked units was that the ratio of  $CF_n$  and  $CF_1$  was often that of two integers (e.g., 3:2, 2:1, 5:2, 3:1,  $\dots$ ), which we considered as having a *harmonic relationship* or being *harmonically related*. For the unit shown in Fig. 2A,  $CF_2$  equals  $2CF_1$ .

Some multi-peaked units had more than two peaks, such as the example shown in Fig. 2B. This unit had three excitatory peaks at 7 kHz ( $CF_1$ ), 10.5 kHz ( $CF_2$ ,  $\sim 1.5CF_1$ ) and 21 kHz ( $CF_3$ ,  $\sim 3CF_1$ ) respectively. There are some common characteristics of multi-peaked units. First, the highest discharge rate evoked by a single tone could occur at any one of the peak frequencies ( $CF_n$ ,  $n = 1, 2, 3, \dots$ ), not necessarily at  $CF_1$ . Second, the minimum response latency could be different at each peak frequency. For example, responses near  $CF_3$  in the unit shown in Fig. 2B had shorter latencies than responses near  $CF_1$  and  $CF_2$ . Third, the rate-level function at each peak frequency could be either monotonic or nonmonotonic. Fourth, response threshold at each peak frequency may differ as well.

An interesting observation of multi-peaked units was that temporal discharge patterns at different peak frequencies were often not identical. By "temporal discharge pattern" we mean the distribution of spike times over a period of time of interest (usually the duration of a stimulus plus a short time period after the stimulus offset) as opposed to the count of spikes or mean firing rate over a period of time of interest. For example, offset responses were observed at  $CF_3$  for the unit shown in Fig. 2B and at  $CF_2$  for the one in Fig. 2C but not at other peak frequencies. Even in the same unit, temporal discharge patterns could vary across sound levels. Figure 3 shows responses of a multi-peaked unit at two sound levels (10 and 40 dB SPL, respectively, Fig. 3, A and B). This unit had one excitatory peak at 10 dB SPL ( $CF_1 = 14.5$  kHz) and three excitatory peaks at 40 dB SPL (21.9, 28.1, and 35.3 kHz, corresponding approximately to  $1.5CF_1$ ,  $2CF_1$ , and  $2.5CF_1$ , respectively) as indicated in Fig. 3C. While there were sustained discharges near 14.5 kHz at 10 dB SPL (Fig. 3A), the unit showed only onset firings at this frequency at 40 dB SPL (Fig. 3B).

### *Population properties of multi-peaked units*

Figure 4A shows the relationship of the lowest response threshold versus  $CF_1$  of 38 multi-peaked units analyzed in this study (open circles). In multi-peaked units, the lowest response threshold could occur at any peak frequency ( $CF_1$ ,  $CF_2$ , or  $CF_3$ ). For comparison, the distribution of response threshold across CF is also shown in Fig. 4A for 113 single-peaked units (pluses) recorded from the same animals. Two distributions are largely overlapping. Most units had low thresholds in the range of 0–20 dB SPL (single-peaked units: 82/113, multi-peaked units: 31/38). We concentrated our sampling in the central area of A1 near the range of frequencies (6–8 kHz) where the first



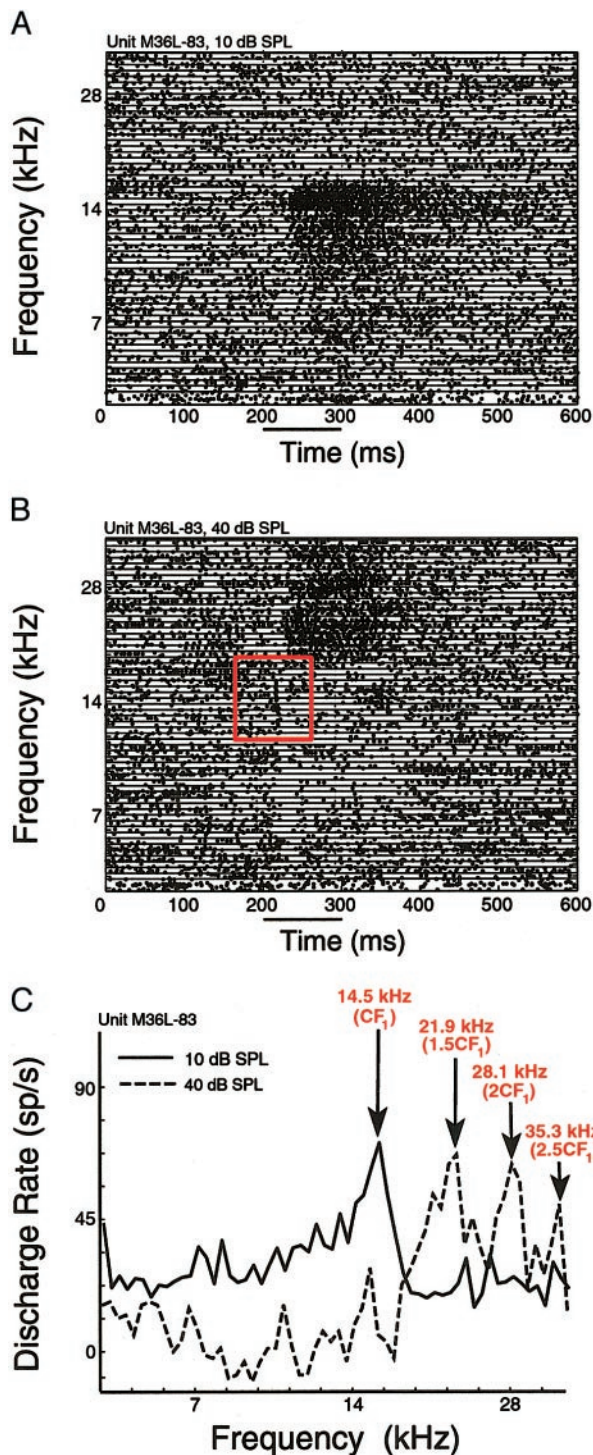


FIG. 3. Example of a multi-peaked unit with different excitatory frequency peaks at different sound levels revealed by single-tone stimulation. Dot raster showing single tone responses at 10 and 40 dB SPL (A and B), respectively. Tones near  $\sim 14.5$  kHz ( $CF_1$ ) elicited strong sustained responses at 10 dB SPL (A) but only onset discharges at 40 dB SPL (B, indicated by a red square). The unit responded to other frequencies at 40 dB SPL with sustained discharges. Discharge rate is plotted vs. tone frequency for the 2 sound levels. C: several frequency peaks were identified. Note that the frequency peaks at both sound levels were harmonically related.

harmonic of many marmoset vocalizations is centered (Agamaite and Wang 1997).

The distribution of peak frequency ratios ( $CF_2/CF_1$ ,  $CF_3/$

$CF_2$ ,  $CF_3/CF_1$ ) for all multi-peaked units is shown in Fig. 4B. A major peak near the ratio of 2 indicates that the most common relationship between the frequency peaks is that of an octave.

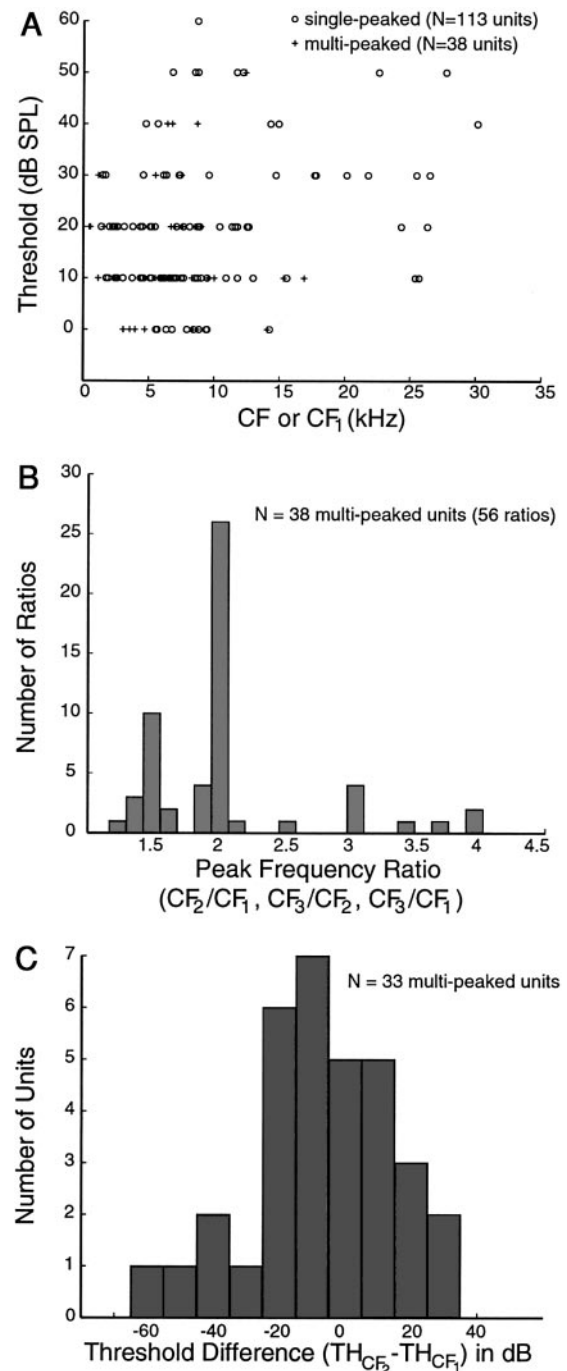


FIG. 4. Single-tone response properties of the single- and multi-peaked units. A: threshold is plotted vs. CF for 113 single-peaked units ( $\circ$ ). The lowest threshold is plotted vs.  $CF_1$  (the lowest frequency peak) for 38 multi-peaked units ( $+$ ). The lowest threshold could be at  $CF_1$ ,  $CF_2$ , or  $CF_3$ . B: distribution of all peak frequency ratios ( $CF_2/CF_1$ ,  $CF_3/CF_1$ ,  $CF_3/CF_2$ ) of multi-peaked units.  $CF_1$ ,  $CF_2$ , or  $CF_3$  are the frequency peaks in multi-peaked units. Because a multi-peaked unit could have  $>2$  peaks, it may be represented by  $>1$  peak frequency ratio in this plot. A total of 56 peak frequency ratios were obtained from 38 multi-peaked units. C: distribution of the threshold difference between  $CF_1$  and the next higher frequency peak ( $CF_2$ ) in multi-peaked units. The threshold at each frequency peak was determined by the rate-level function measured at that peak frequency.

The second largest peak in Fig. 4*B* is at the ratio of 1.5 (3/2) or half octave step. For 33 multi-peaked units, we were able to determine the response threshold at all frequency peaks. Figure 4*C* shows the distribution of the threshold differences between response peaks at CF<sub>2</sub> and that at CF<sub>1</sub>. In 18 of 33 units, thresholds at higher peak frequencies (CF<sub>2</sub>) were lower than that at CF<sub>1</sub>. The opposite was observed in 10 of 33 units. The remaining five units had approximately equal thresholds at CF<sub>1</sub> and CF<sub>2</sub>.

### *Two-tone facilitation in multi-peaked units*

A natural question for multi-peaked neurons is whether their responses are facilitated when their multiple excitatory regions are simultaneously stimulated. For example, the unit shown in Fig. 2*A* has two harmonically related peaks at ~10 (CF<sub>1</sub>) and 20 kHz (CF<sub>2</sub>). We played the tones at these two peak frequencies simultaneously, fixing the frequency of the first tone (S1) at CF<sub>1</sub> and varying the frequency of the second tone (S2) from 800 Hz to 30 kHz (in 36 logarithmic steps, Fig. 5, *A* and *B*), both tones were delivered at 50 dB SPL. There were clear modulations by the S2-tone at various frequencies (Fig. 5, *A* and *B*). At frequencies near CF<sub>2</sub>, the unit's response was facilitated, with the strongest facilitation at CF<sub>2</sub>. The two-tone stimulation also revealed flanking inhibitory regions near CF<sub>1</sub> that have been described in detail by others (Shamma and Symmes 1985; Shamma et al. 1993; Suga 1965a,b; Sutter et al. 1999) as a part of the response area of an A1 neuron. There are two features to note about the facilitation near CF<sub>2</sub> as shown in the dot raster in Fig. 5*A*. First, the temporal profile of responses at CF<sub>2</sub> is different from that at CF<sub>1</sub>: the latency is a little shorter at CF<sub>2</sub> and the response is sustained for a longer period of time. Second, there are clear offset responses at CF<sub>2</sub> but little at CF<sub>1</sub>.

The two-tone facilitation was generally dependent on the sound level of the S2-tone. Figure 5, *C* and *D*, shows responses of the same unit as described in Fig. 5, *A* and *B*, when the CF<sub>2</sub> sound level was varied between 10 and 80 dB SPL, while CF<sub>1</sub> sound level was fixed at 60 dB SPL. Differences in temporal response profile are apparent in the PSTHs (Fig. 5*C*). In particular, discharges lasted longer for S2 at 50–80 dB SPL. The corresponding rate-level function for the two-tone presentation is shown in Fig. 5*D*, where it is compared with the rate-level functions of CF<sub>1</sub> and CF<sub>2</sub> tones played separately. A line representing a linear summation is shown by adding the neural response at CF<sub>1</sub> of 60 dB SPL to the rate-level function of the CF<sub>2</sub> tone. It is evident that the two-tone interactions produced nonlinear facilitation at suprathreshold sound levels, where the responses to the two tones played together were stronger than the sum of the responses to both tones played individually.

In Fig. 5, *E* and *F*, we explore further two-tone interactions at different sound levels in the same unit. Figure 5*E* shows the two-tone responses with CF<sub>2</sub> tone at 10 dB and CF<sub>1</sub> varying from 10 to 80 dB SPL. Although the CF<sub>2</sub> tone by itself evoked no response, adding it to the CF<sub>1</sub> tone increased the responses at all sound levels tested, as compared with the responses to the CF<sub>1</sub> tone alone. This indicates that adding a harmonic, sub-threshold tone can lower response threshold and enhance two-tone responses across sound levels in a multi-peaked unit. Figure 5*F* shows the results from an experiment in which the roles of CF<sub>1</sub> and CF<sub>2</sub> were switched. Here, we fixed the CF<sub>1</sub> sound level at 10 dB SPL and varied the CF<sub>2</sub> sound level from

10 to 80 dB SPL. Two-tone responses again showed facilitation as compared with the rate-level function of CF<sub>2</sub> tone alone, even though CF<sub>1</sub> tone at 10 dB SPL did not produce any response. Furthermore, the rate-level function of the two-tone response has the similar nonmonotonic shape as the rate-level function of CF<sub>2</sub> tone (Fig. 5*F*).

In 25 of 38 multi-peaked units, we were able to carry out tests of two-tone influences. In most nonmonotonic units, we tested two-tone properties near the peak of the rate-level function of S1 tone to determine if there was a facilitatory response. The distribution of the percent facilitation measured in these units is shown in Fig. 6. A percent facilitation of 100% means that the two-tone responses are twice as strong compared with the single-tone responses. The properties of the two-tone facilitation for a wide range of CF<sub>1</sub> frequencies tested indicate the generality of this mechanism at work in A1 neurons.

### *Facilitation by distant off-CF tones in single-peaked units*

Using the two-tone paradigm, we observed in single-peaked units, facilitation by the second tone (S2) placed outside of a unit's central excitatory area and flanking inhibitory regions. We refer to this type of two-tone facilitation as the facilitation by *distant* off-CF tones. Two examples of such responses are shown in Fig. 7. The unit shown in Fig. 7, *A* and *B*, has a CF of 4.53 kHz, a threshold of 20 dB SPL and a preferred level of 40 dB SPL. Figure 7, *A* and *B*, shows the result from a two-tone test for this unit, representative of the tests given to other units. The two-tone responses were obtained for a range of S2 frequency (0.35–13 kHz). The two-tone paradigm revealed flanking inhibitory regions at frequencies near the CF (Fig. 7*A*). The reduction of discharge rate at CF was due to nonmonotonic rate-level characteristics of this unit (Fig. 7*B*). There were facilitatory responses away from the CF near a harmonically related frequency (7 kHz, ~1.5 times CF), where the two-tone responses were much stronger (~150%) than the single-tone response at CF (Fig. 7*B*). Note that the frequency of this facilitatory peak is outside the flanking inhibitory regions of the unit. In the single-tone test, there were no responses at these distant frequencies. Moreover, two-tone facilitation was also observed at several other higher frequencies (e.g., near 11.4 kHz) though much smaller in magnitude than the facilitatory responses near 7 kHz (Fig. 7, *A* and *B*). An interesting feature of the two-tone facilitation with the S2 tone near 7 kHz was that it resulted from an increase of sustained discharges (Fig. 7*A*). The response was largely onset when the S1 tone was played alone.

Figure 7, *C* and *D*, show data from another representative unit. The two-tone responses (Fig. 7*D*) revealed best facilitation with the S2 tone near 3 kHz (~2 times CF), where discharge rates were ~250% higher than the single tone response at CF. This facilitatory region is outside the flanking inhibitory region. The temporal profile of the two-tone facilitatory responses near 3 kHz differed from that of the single-tone response in that response latencies were much shorter and sustained responses were much stronger (Fig. 7*C*).

### *Distant inhibitory influences from outside the response area*

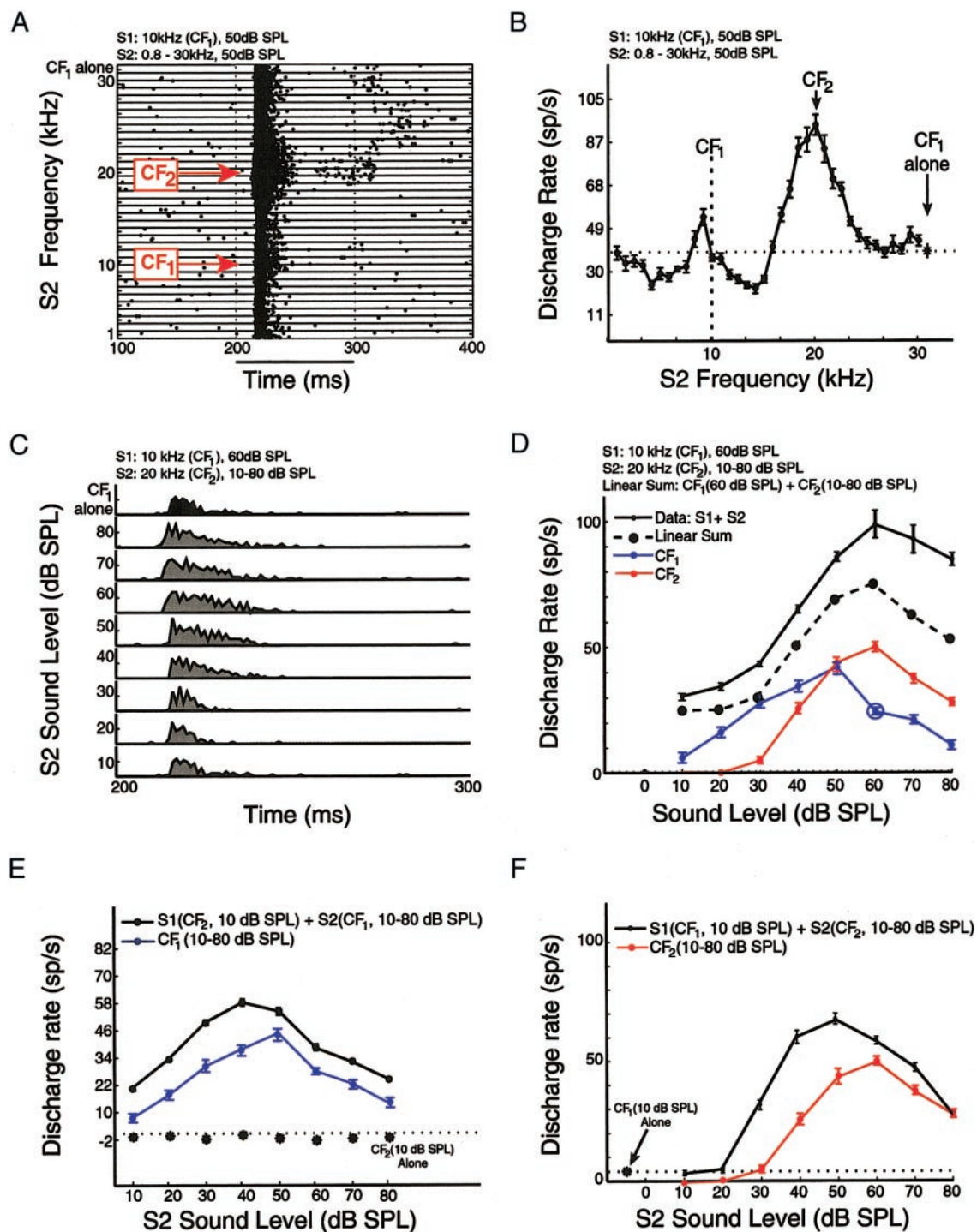
Because spontaneous rates of most A1 neurons are generally low, it is not always possible to observe inhibitory side bands



using single tone stimuli. Inhibitory response areas flanking the CF-centered excitatory response area could be revealed using simultaneously played two tones. In addition to the flanking inhibition, we observed inhibition at distant frequencies outside the response areas of a neuron. Figure 8 depicts three representative units in which an S2 tone at a distant frequency elicited inhibition. The unit shown in Fig. 8A had a CF of 4.42 kHz and was inhibited by S2 tones at frequencies near  $2 \times \text{CF}$ . Sometimes inhibitory influences were observed at frequencies further than one octave away from CF, as shown by the example in Fig. 8B. This unit had a CF of 12.25 kHz. The S2

tone at  $3 \times \text{CF}$  completely inhibited the response to the CF-tone (Fig. 8B). The example in Fig. 8C illustrates that in some single-peaked units, both facilitation and inhibition were observed at different S2 frequencies. This unit had a CF of 14.7 kHz and was inhibited at a harmonically related frequency ( $0.5 \times \text{CF}$ ) but facilitated at another frequency (17.2 kHz).

The examples shown in Figs. 7 and 8 demonstrated both facilitatory and inhibitory influences from outside of the response area, often observed in the same unit. As is evident from some of these examples, in addition to changes in discharge rate, two-tone interactions also altered temporal dis-





charge patterns (Fig. 7, *A* and *C*). Two additional examples shown in Fig. 9 further demonstrate this point. In Fig. 9*A*, two-tone responses revealed both facilitatory and inhibitory regions away from CF. Interestingly, a new, short-latency onset response was observed in two-tone conditions for S2 frequencies in the range of 6–7 kHz (indicated by a red square in Fig. 9*A*). This onset component did not exist at any frequency in single-tone conditions. Figure 9*B* shows a unit that had strong facilitation at S2 frequencies near 1.5\*CF. The facilitation was manifested by increased sustained discharges near the facilitatory frequencies (Fig. 9*B*).

#### *Distribution of spectral integration peaks in population of single-peaked units*

A total of 76/113 single-peaked units showed some forms of response modulation (facilitation or inhibition) by S2 tones distant from the CF of a unit. In 51/113 units, we were able to determine a locus of facilitation distant from CF. In a separate but overlapping population of units (51/113), we were able to determine a locus of inhibition distant from CF. Both distant facilitation and inhibition were observed in 27/113 units. These numbers are probably an underestimate of the extent of two-tone modulations in single-peaked A1 units because it was not always feasible to study each unit with all possible combinations of two-tone frequencies and intensities.

Figure 10*A* shows the distribution of best facilitatory peaks in single-peaked units (see METHODS). In some units, the facilitation peaks had harmonic relationships to the CF (0.5\*CF, 1.5\*CF, etc.), while in others nonharmonically related frequencies evoked facilitation. Figure 10*B* shows distribution of best inhibitory peaks in single-peaked units (see METHODS). Some units (26/51 = ~51%) had distant inhibition at harmonically related frequencies (0.5\*CF, 1.5\*CF, 2\*CF, 3\*CF), most noticeably at 0.5\*CF and 2\*CF (Fig. 10*B*). Data in Fig. 10, *A* and *B*, represent the most prominent facilitation and inhibition in each unit, respectively. In some units, there was more than one

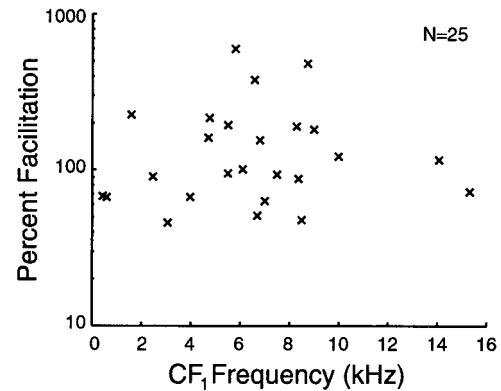


FIG. 6. Summary of 2-tone facilitation evaluated in 25 multi-peaked units. The percent facilitation (y axis), the largest 2-tone facilitation found in each unit using the 2-tone protocol, is plotted vs. the CF<sub>1</sub> frequency of each multi-peaked unit. The magnitude of the 2-tone facilitation is measured by positive percent change in discharge rate (see METHODS).

facilitatory or inhibitory peak at distant S2 frequencies. Frequency for these secondary peaks were calculated in these units and included in the overall distribution of all modulatory influences (139 peaks—facilitatory and inhibitory) in Fig. 10*C*. There are three prominent peaks in this distribution at 0.5\*CF, 2\*CF, and 3\*CF, respectively.

#### *Sound level dependency of two-tone facilitation and inhibition*

The two-tone facilitation and inhibition were found to depend on sound levels of both S1 and S2 tones. In general, this sound level dependency was closely related to the characteristics of rate-level functions of S1 and S2 tones. The preferred sound levels at CF<sub>1</sub> and CF<sub>2</sub> frequencies were often not the same. The unit shown in Fig. 11 responded after stimulus onset at 20–30 dB SPL to CF<sub>1</sub> tone (2.46 kHz), but it responded after stimulus offset to CF<sub>2</sub> tone (4.92 kHz) at higher sound levels

FIG. 5. Examples of 2-tone responses in a multi-peaked unit, the same unit as shown in Fig. 2*A* (unit M28494-199). In all the plots showing 2-tone responses, the following convention is used: each 2-tone pair is defined by S1 (stimulus 1, fixed tone) and S2 (stimulus 2, variable tone). The specific parameters for S1 or S2 are indicated on each plot. *A*: dot raster showing 2-tone responses for S1 at CF<sub>1</sub> (10 kHz), 50 dB SPL and S2 at 0.8–30 kHz (in linear steps of ~800 Hz), 50 dB SPL. The stimulus duration is indicated by a thick bar under the horizontal axis. Each 2-tone stimulus was delivered 20 times in random order. Responses at the control condition (CF<sub>1</sub> tone alone at 50 dB SPL) are shown on the top row of the plot. *B*: discharge rate is plotted vs. S2 frequency for the data shown in *A*. Discharge rates were calculated over the duration of the stimuli (100 ms). The error bars in this and other similar plots hereafter represent the standard errors of the means (SE). The 2-tone responses show both facilitation and inhibition at various frequencies away from CF<sub>1</sub>. Note that the 2-tone facilitation is strongest at an S2 frequency near 20 kHz (CF<sub>2</sub> of this unit). The 2-tone responses also reveal inhibitory regions below CF<sub>1</sub> and between CF<sub>1</sub> and CF<sub>2</sub>. The asterisk and horizontal dotted line indicate the discharge rate of responses to CF<sub>1</sub> tone alone. *C*: PSTHs showing 2-tone responses over a range of sound levels of S2 tone (at CF<sub>2</sub>, 20 kHz). The sound level of S1 tone (at CF<sub>1</sub>, 10 kHz) was kept constant at 60 dB SPL. Responses at the control condition (CF<sub>1</sub> tone alone at 60 dB SPL) are shown on the top row of the plot. *D*: various rate-level functions are shown for 1- and 2-tone responses. The 2-tone data (black solid line with small circles) were obtained with S1 (CF<sub>1</sub>) tone fixed at 60 dB and S2 (CF<sub>2</sub>) tone varying from 0 to 80 dB SPL. The linear summation curve (black dashed line with large circles) is calculated by adding the discharge rate of the response to CF<sub>1</sub> tone alone at 60 dB SPL (blue, indicated by an open circle) to the rate-level function of the response to CF<sub>2</sub> tone alone (red solid line with circles). The strongest 2-tone facilitation was observed at 60 dB SPL, which is the peak of the rate-level function for CF<sub>2</sub> tone alone. *E*: comparison between 1- and 2-tone rate-level functions when sound level of CF<sub>1</sub> tone is varied. The 2-tone rate-level function (black line with circles) was obtained with S1 (CF<sub>2</sub>) tone set at a subthreshold sound level (10 dB SPL) and the sound level of the S2 (CF<sub>1</sub>) varying from 10 dB to 80 dB SPL. The 1-tone rate-level function (blue line with circles) was obtained with the CF<sub>1</sub> tone presented alone (10–80 dB SPL). The asterisks indicate responses to the CF<sub>2</sub> tone (10 dB SPL) played alone at each 2-tone condition. Note that the addition of the subthreshold harmonic tone (CF<sub>2</sub>) increased the discharge rate to the CF<sub>1</sub> tone at all tested sound levels. *F*: comparison between 1- and 2-tone rate-level functions when sound level of CF<sub>2</sub> tone is varied. The 2-tone rate-level function (black line with circles) was obtained with S1 (CF<sub>1</sub>) tone set at a subthreshold sound level (10 dB SPL) and the sound level of the S2 (CF<sub>2</sub>) varying from 10 dB to 80 dB SPL. The 1-tone rate-level function (red line with circles) was obtained with the CF<sub>2</sub> tone presented alone (10–80 dB SPL). The asterisk and horizontal dotted line indicate responses to the CF<sub>1</sub> tone (10 dB SPL) played alone. Note that the addition of the subthreshold CF<sub>1</sub> tone resulted in a lower threshold for the CF<sub>2</sub> tone (~10 dB shift) and facilitation across most tested sound levels of the CF<sub>2</sub> tone.

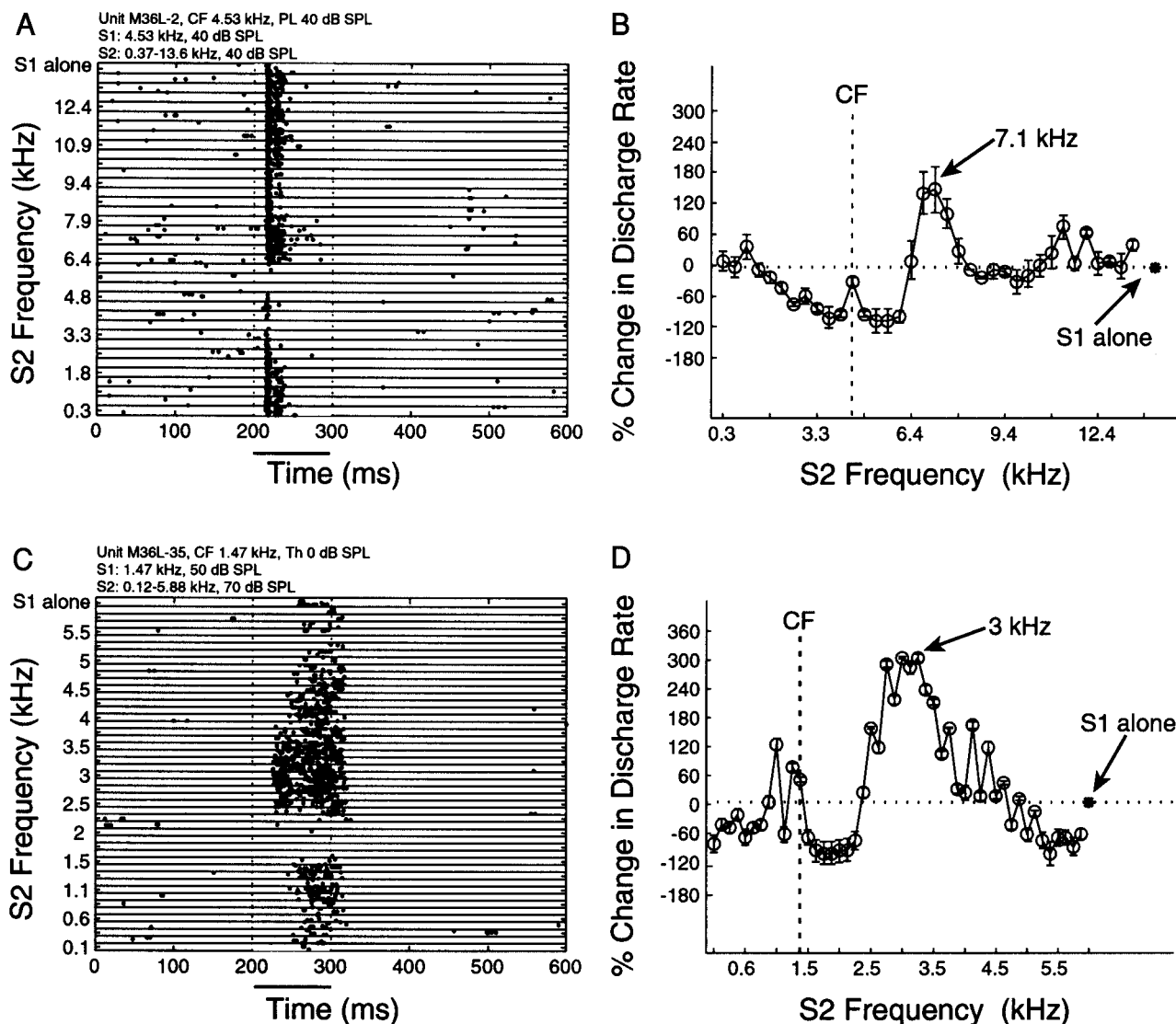


FIG. 7. Examples of 2-tone responses in 2 representative single-peaked units (*A* and *B* and *C* and *D*, respectively). Each 2-tone pair is defined by S1 (stimulus 1, fixed tone) and S2 (stimulus 2, variable tone). The specific parameters for S1 or S2 are indicated on each plot. *A*: dot raster shows 2-tone responses recorded from the same unit as shown in Fig. 1. This unit has a nonmonotonic rate-level function at CF with the preferred sound level (PL) at 40 dB SPL. The 2-tone responses were obtained with S1 at CF (4.53 kHz), 40 dB SPL and S2 at 0.37–13.6 kHz, 40 dB SPL (in linear steps of  $\sim 377$  Hz, see METHODS). A subset of the S2 frequencies is marked on the ordinate. The stimulus duration (100 ms) is indicated by a thick bar under the horizontal axis. Responses at the control condition (S1 tone alone at 40 dB SPL) are shown on the top row of the plot. *B*: percent change in discharge rate (see METHODS) is plotted vs. S2 frequency for the data shown in *A*. Discharge rates were calculated over the duration of the stimuli (100 ms). The asterisk and horizontal dotted line indicate the discharge rate of responses to the S1 tone alone. The strongest facilitation was seen at an S2 frequency of  $\sim 7.1$  kHz (pointed by an arrow). *C*: same format as in *A*. This unit has a monotonic rate-level function at CF with the threshold (Th) at 0 dB SPL. S1 was at CF (1.47 kHz), 50 dB SPL and S2 at 0.12–5.88 kHz, 70 dB SPL (in linear steps of  $\sim 122$  Hz). *D*: same format as in *B* for the data shown in *C*.

(70–80 dB SPL; Fig. 11*A*). This offset response is an indication of release from inhibition that keeps the unit from responding to the CF<sub>2</sub> tone at higher sound levels during the stimulus. We tested this unit over a range of CF<sub>2</sub> sound levels using the two-tone paradigm with the CF<sub>1</sub> tone set at its best level of 70 dB SPL (Fig. 11*B*). The strongest facilitatory responses with the shortest latencies were observed when the CF<sub>1</sub> and CF<sub>2</sub> tones were played together at their respective preferred sound levels (Fig. 11*B*). The two-tone response as a function of CF<sub>2</sub> sound levels (Fig. 11*C*) mirrors the rate-level function of the CF<sub>2</sub> tone alone (Fig. 11*A*). The two tones evoked facilitatory responses for S2 tones at 20–30 dB SPL but inhibitory re-

sponses for S2 tones at 50–80 dB SPL. This example shows that facilitatory or inhibitory interactions for two-tone paradigms are dependent on the sound levels of both tones and that when both tones are played at their respective preferred levels, the maximum facilitation is evoked.

Figure 12 shows the dependency of facilitation and inhibition on the sound level of two tones in another representative multi-peaked unit. This unit had two frequency peaks at 8.37 kHz (CF<sub>1</sub>) and 16.7 kHz (CF<sub>2</sub>), respectively. The rate-level functions at both frequencies were nonmonotonic (Fig. 12*A*). We tested two two-tone paradigms for this unit. In Fig. 12*B*, the CF<sub>1</sub> tone was played at 40 dB SPL (near the peak of its



rate-level function, Fig. 12A) together with the CF<sub>2</sub> tone at varying sound levels (0–80 dB). The two-tone response, largely facilitatory (Fig. 12B), follows the shape of the CF<sub>2</sub> rate-level function (Fig. 12A). In Fig. 12C, the CF<sub>2</sub> tone was played at 50 dB SPL (near the peak of its rate-level function, Fig. 12A) together with the CF<sub>1</sub> tone at varying sound levels (0–80 dB). The two-tone response profile showed a nonmonotonic shape similar to the CF<sub>1</sub> rate-level function (Fig. 12A). Two-tone responses were facilitatory for CF<sub>1</sub> tones at 20–30

dB SPL but inhibited as compared with the one-tone condition when the CF<sub>1</sub> tone was at 80 dB SPL (Fig. 12C). This example also demonstrates a general observation, i.e., the changes in two-tone responses over sound level closely follow the rate-level characteristics of the second tone that presumably drive the modulatory inputs to the unit.

The dependency of two-tone facilitation on S2 sound level described for multi-peaked units in Figs. 11 and 12 was also observed in many single-peaked units. A representative example is shown in Fig. 13 (A and B) (the same unit as shown in Fig. 7, C and D). The S1 tone (at CF) and the S2 tone (at 2\*CF) were played simultaneously with the S2 sound level varying from 30 to 80 dB SPL. The two-tone facilitation was observed over a specific range of S2 sound levels (60–80 dB SPL; Fig. 13B). Additional examples of two-tone facilitatory responses as functions of the S2 sound level are shown in Fig. 13C. In most cases when we were able to determine a range of frequencies away from CF for which two-tone stimulation resulted in facilitation, the facilitation was highly dependent on the sound level of the S2 tone.

#### Temporal aspects of two-tone facilitation

As the examples in the previous figures for both single- and multi-peaked units have shown, responses to two-tone stimuli may include up to three components: onset, sustained, and offset discharges. Given the variety in the temporal discharge patterns, it is important to know how far two tones could be separated in time and still have interactions to modify neural responses. We examined this issue in a subset of units by varying the delay between the onsets of S1 and S2 tones. A typical example of a single-peaked unit is shown in Fig. 14A. Responses to two simultaneously presented tones (with the S2 frequency varying) for this unit were shown in Fig. 7, A and B. The onset delay was varied over a range of 0–125 ms. At the onset delay of 25 ms, the S2 tone (at a facilitatory off-CF frequency) started 25 ms before the S1 tone (at CF of the unit) (Fig. 14Aa). The durations for both tones were 100 ms. The two tones were completely overlapping at the onset delay of 0 ms, and nonoverlapping at onset delays of  $\geq 100$  ms. Typically in such experiments, we also recorded the control response (S1 alone) with each experimental condition (S2 with delayed S1). Figure 14Ab shows the percentage of change in discharge rate as a function of the onset delay. The results indicate that the two tones have to overlap to some extent in time to achieve any

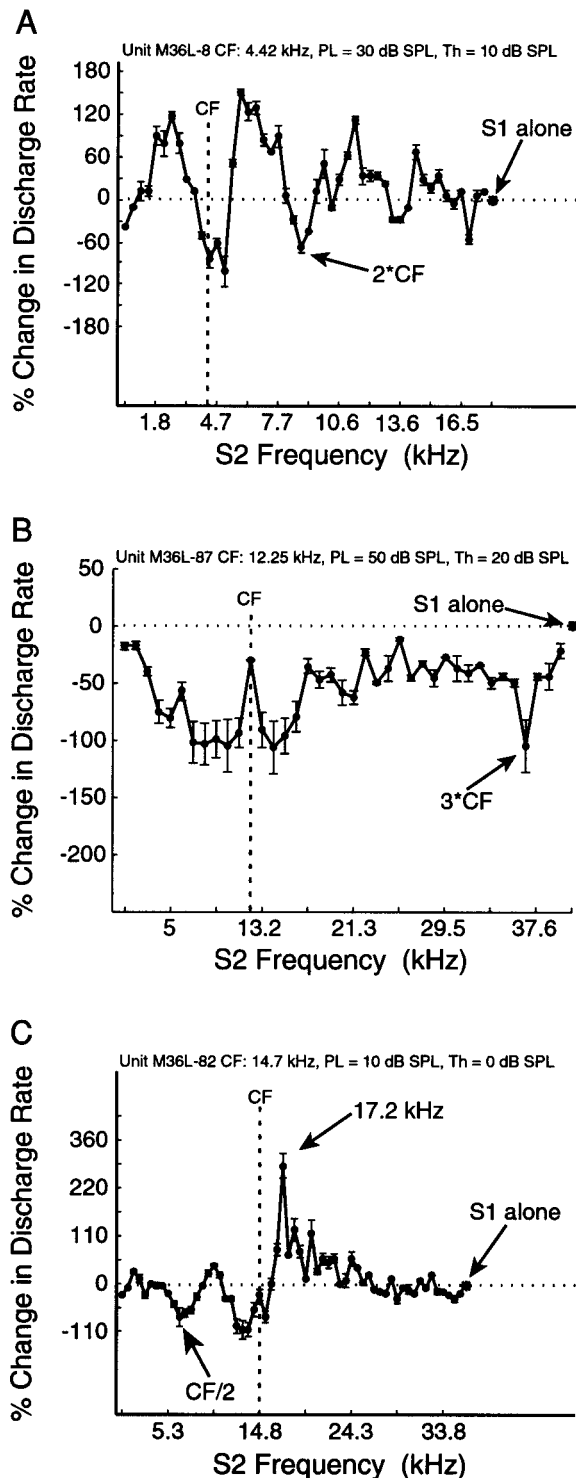


FIG. 8. Two-tone responses from 3 representative single-peaked units showing distant off-CF inhibitions. All 3 units had nonmonotonic rate-level functions from which a preferred sound level and threshold were calculated (indicated on each plot). \* and  $\cdots$ , the discharge rate of responses to the S1 tone alone. A: 2-tone responses recorded from a single-peaked unit with S1 at CF (4.42 kHz), 30 dB SPL and S2 at 0.3–17.6 kHz and 30 dB SPL (in linear steps of  $\sim 300$  Hz). Percent change in discharge rate is plotted vs. S2 frequency. The strongest distant off-CF inhibition was at 8.8 kHz (indicated by an  $\rightarrow$ ). B: same format as in A for another single-peaked unit. S1 was at CF (12.25 kHz), 50 dB SPL and S2 at 1–39.6 kHz, 40 dB SPL (in linear steps of  $\sim 1,000$  Hz). The strongest distant off-CF inhibition was at 3\*CF (indicated by an  $\rightarrow$ ). C: same format as in A for another single-peaked unit. S1 was at CF (14.7 kHz), 30 dB SPL and S2 at 0.59–35.6 kHz, 30 dB SPL (in linear steps of  $\sim 590$  Hz). The strongest distant inhibition was near CF/2 (indicated by an  $\rightarrow$ ), whereas the strongest facilitation was evoked at 17.2 kHz (indicated by an  $\rightarrow$ ). The inhibitory region near and below CF was considered part of the classical receptive field.

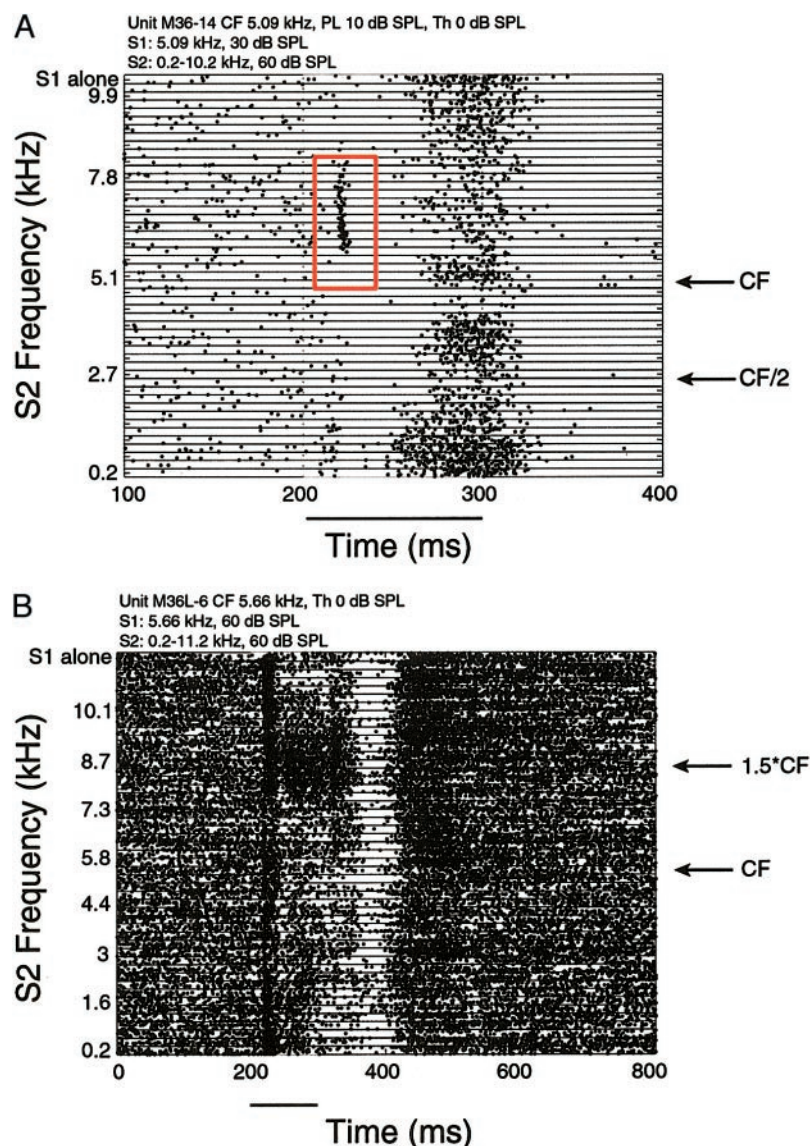


FIG. 9. Two-tone responses showing changes in temporal discharge patterns due to stimuli outside the classical receptive field. **A**: 2-tone responses of a single-peaked unit with S1 at CF (5.09 kHz), 30 dB SPL and S2 at 0.2–10.2 kHz, 60 dB SPL (in linear steps of ~200 Hz). A red square indicates emerged short latency, onset components under certain 2-tone conditions. Note the distant inhibitory area near CF/2 (indicated by an arrow). This unit had a nonmonotonic rate-level function with a preferred sound level of 10 dB SPL and threshold of 0 dB SPL. **B**: 2-tone responses of a single-peaked unit with S1 at CF (5.66 kHz), 60 dB SPL and S2 at 0.2–11.2 kHz, 60 dB SPL (in linear steps of ~230 Hz). Note that the strongest facilitation is near 8.5 kHz (1.5\*CF). This unit had a monotonic rate-level function with a threshold of 0 dB SPL.

facilitation and that the facilitation becomes weaker as the onset delay increases.

Figure 14*B* shows an example of a multi-peaked unit. When both CF<sub>1</sub> and CF<sub>2</sub> tones (50 ms in duration) were played simultaneously, there was a strong facilitation. The single-tone response in this unit ended within the first 25–30 ms after the tone onset (Fig. 14*Ba*). When the onset delay between the two tones was <25–30 ms (i.e., the S1 tone was played before the response to the S2 tone was ended), two-tone facilitation was observed. Once the response to the S2 tone ended, the two-tone responses were weaker than the response to the S1 tone alone (Fig. 14*Bb*). In all the units where we tested the effects of varying the onset delay, the maximum two-tone response was always observed when S1 and S2 tones were delivered simultaneously, as demonstrated in the two examples in Fig. 14.

## DISCUSSION

This study demonstrated excitatory and inhibitory modulations in A1 neurons arising from tones outside of the classical receptive field (defined by a central excitation area

surrounded by flanking inhibitory areas). Approximately 20% of the units in marmoset A1 were found to have multi-peaked tuning characteristics. The distribution of peak frequency ratios (e.g., CF<sub>2</sub>/CF<sub>1</sub>) showed maxima at 1.5 and 2 (Fig. 4), indicating a harmonic structure in the multi-peaked A1 units. One of the functional properties that characterized units with multi-peaked tuning characteristics was the facilitation in the response when these units were stimulated by two tones at peak frequencies with appropriate combinations of sound levels. Two-tone facilitation was strongest when the two tones were played nearly simultaneously. All units with multi-peaked tuning characteristics that were tested for two-tone interactions exhibited facilitatory responses. We also found that responses of A1 units with single-peaked tuning characteristics (~80% of samples) could be modulated by tones over a wide range of frequencies away from CF. In the population of single-peaked units, the harmonic structure observed in two-tone interactions was closely linked to inhibition rather than facilitation (Fig. 10).



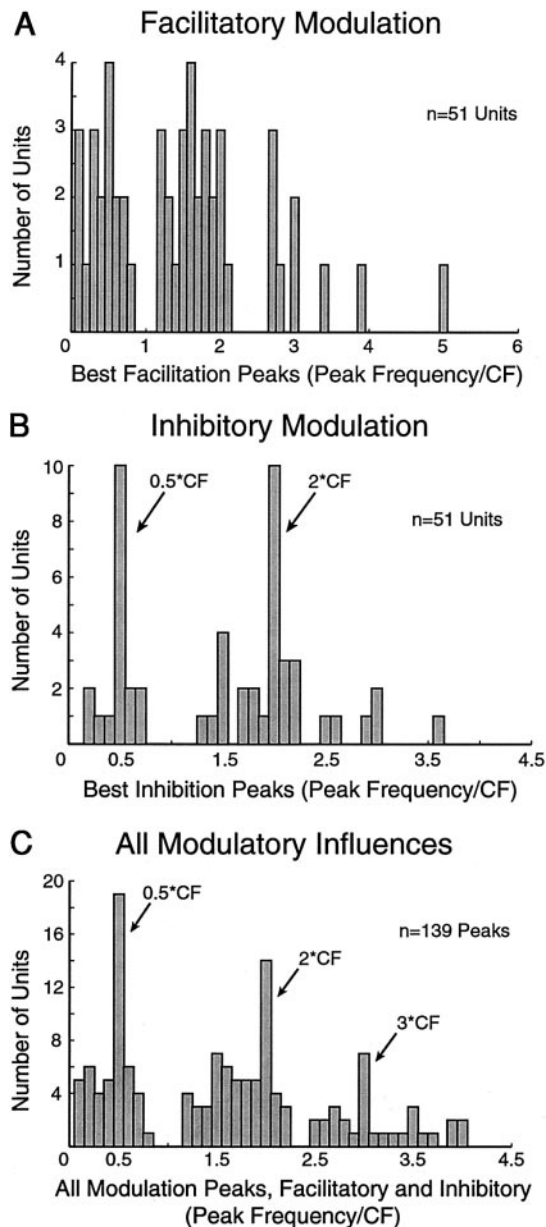


FIG. 10. Response modulations in the population of single-peaked unit. *A*: distribution of major facilitatory peaks (see METHODS) in 51 of 113 single-peaked units. *B*: distribution of major inhibitory peaks in 51 of 113 single-peaked units. The units included in this plot partially overlap with the group of units included in *A*. Neurons in A1 have inhibitory influences from a wide range of frequencies outside the receptive fields, predominantly from harmonically related frequencies ( $0.5 \times CF$ ,  $2 \times CF$ ). *C*: distribution of all modulatory peaks measured from 76 of 113 units that showed facilitation and/or inhibition in their 2-tone responses. There were a total of 139 measured peaks.

#### Technical considerations

Because unwanted harmonics are commonly generated by nonlinearity in acoustic systems, it is important to rule out the possibility of these artifacts in the observations of multi-peaked tuning characteristics. We tested our sound delivery system thoroughly and found that harmonics were  $\geq 43$  dB lower than the fundamental for tones delivered at the highest sound level used in this study (80 dB SPL). As sound level decreased, the magnitudes of the harmonics dropped at rates faster than the fundamental. Moreover, several lines of evidence from our

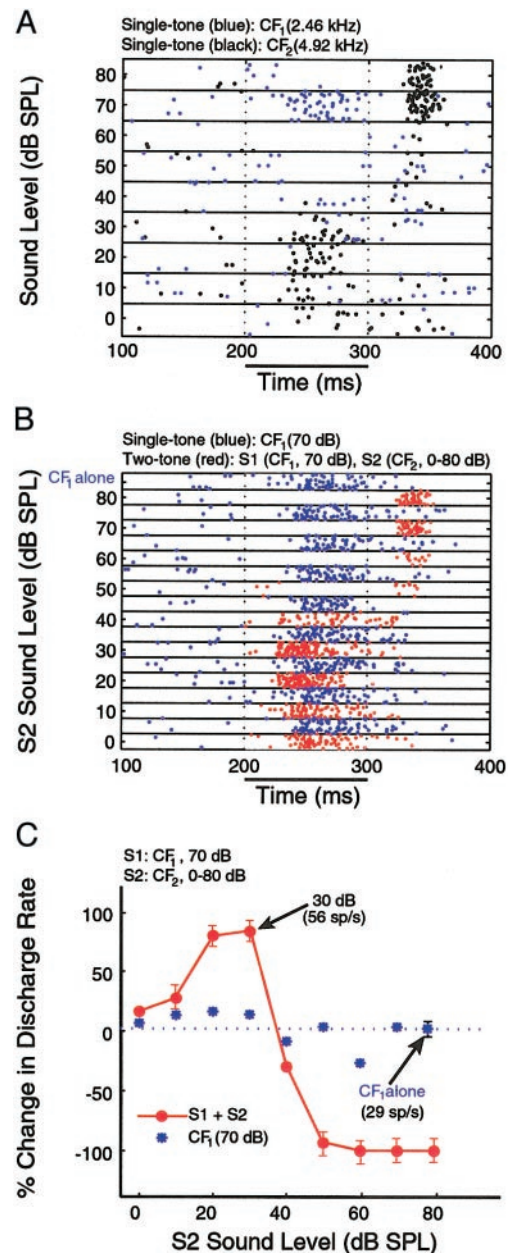


FIG. 11. Sound level dependency of 2-tone facilitation and inhibition is illustrated in a multi-peaked unit (*unit M36L-4*). This unit was tuned to 2 frequencies that are harmonically related ( $CF_1 = 2.46$  kHz and  $CF_2 = 4.92$  kHz). *A*: dot raster showing responses to single-tones placed at  $CF_1$  (blue dots) or  $CF_2$  (black dots) with sound level varying from 0 to 80 dB SPL. The stimulus duration is indicated by a thick bar under the *x* axis. Note that responses to  $CF_1$  and  $CF_2$  tones peaked at 70 and 20–30 dB SPL, respectively. *B*: each alternate block (red) represents a 2-tone response with S1 at  $CF_1$  (70 dB SPL) and S2 at  $CF_2$  (0–80 dB SPL), whereas the other blocks (blue) represent single-tone responses to  $CF_1$  tone alone (70 dB SPL). *C*: percent change in discharge rate is plotted vs. S2 sound level based on the 2-tone data shown in *B* (red). For comparison, responses to  $CF_1$  tone played alone at each 2-tone condition are also shown (blue). Discharge rates at the peak of the 2-tone curve and at the  $CF_1$  alone condition are indicated on the plot. The discharge rates were calculated over the stimulus duration. Note that the 2-tone responses were facilitated with the S2 tone at 20–30 dB SPL but inhibited at higher sound levels of the S2 tone (50–80 dB SPL). The strongest facilitation was achieved when both  $CF_1$  and  $CF_2$  tones were played at their respective preferred sound levels. Response latencies were also the shortest under this condition (*B*).

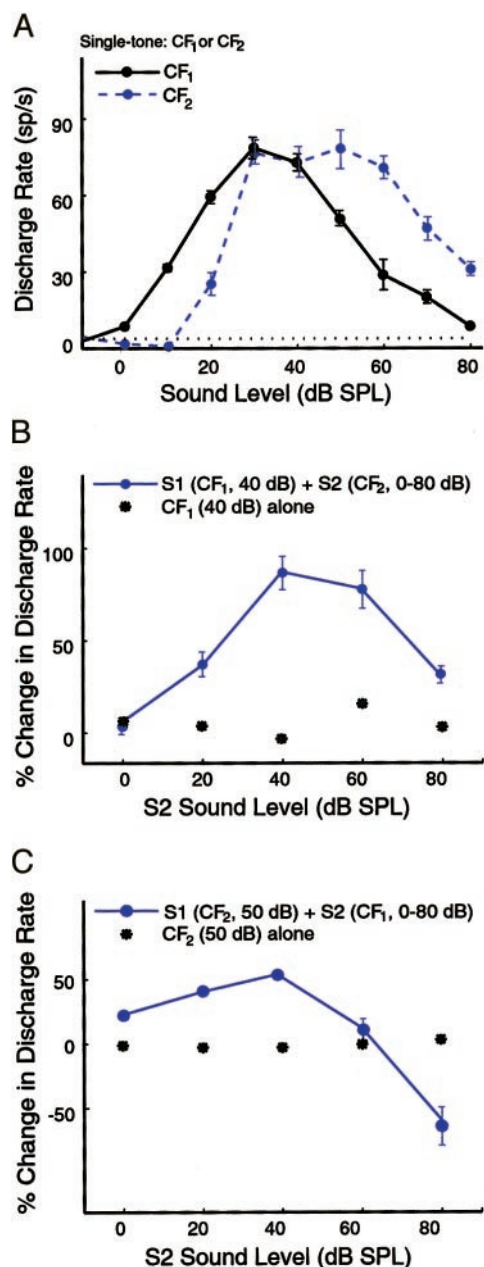


FIG. 12. Interactions between 2 tones at different sound levels. This multi-peaked unit (unit M36L-73) had 2 excitatory peaks (CF<sub>1</sub> = 8.37 kHz and CF<sub>2</sub> = 16.7 kHz). **A**: single-tone rate-level functions for CF<sub>1</sub> (black) and CF<sub>2</sub> (blue) tones, respectively. **B**: 2-tone rate-level function with S1 at CF<sub>1</sub> (40 dB, near the preferred sound level of CF<sub>1</sub> rate-level function, see **A**) and S2 at CF<sub>2</sub> (0–80 dB SPL). For comparison, responses to CF<sub>1</sub> tone (40 dB SPL) played alone at each 2-tone condition are also shown (\*). Note that the maximum facilitation was achieved for S2 near 40 dB SPL. **C**: 2-tone rate-level function with S1 at CF<sub>2</sub> (50 dB, near the preferred sound level of CF<sub>2</sub> rate-level function, see **A**) and S2 at CF<sub>1</sub> (0–80 dB SPL). For comparison, responses to CF<sub>2</sub> tone (50 dB SPL) played alone at each 2-tone condition are also shown (\*). Note that the response was inhibited by S2 tone at 80 dB SPL, but facilitated at other sound levels (0–40 dB SPL).

data argued against the possibilities of artifacts. First, only a subset of units recorded in our experiments had multi-peaked receptive fields (~20% of samples). The response thresholds of these units were comparable to the thresholds of the single-peaked units (Fig. 4A). Nonlinearities generated by our stimulus delivery system should affect all units with similar re-

sponse thresholds. Second, temporal discharge patterns produced by single tones at different frequency peaks in the same unit were generally different (e.g., Figs. 2 and 3). Harmonic artifacts should result in temporal discharge patterns similar to that produced by the fundamental component. Third, temporal profiles of two-tone responses can be different (e.g., having longer sustained components or new offset responses, Figs. 7 and 9) from temporal profiles of single-tone responses. Fourth, differences in thresholds between frequency peaks were as little as 0 dB and no more than 20 dB in most multi-peaked units (Fig. 4C). Larger threshold differences would be expected if acoustic nonlinearity contributed to the observed multi-peaked tuning characteristics.

The statistics for the occurrence of multi-peaked units (~20% in our study) have some experimental biases that are worth mentioning. We recorded from A1 neurons indiscriminately. That is, we studied every unit from which we could record stably. While we tried to record from the full extent of A1, we may have missed parts of A1 where these multi-peaked units might be more or less concentrated. However, our samples of multi- and single-peaked units appeared to be distributed over a wide range of CFs (Fig. 4A). We chose the awake marmoset as our experimental model to avoid confounding effects of anesthetics. In the reported experiments, the animals were not required to perform behavioral tasks when the acoustic stimuli were delivered. Whether the two-tone responses characterized in our study are affected by different behavioral states, especially when an animal attends to or discriminates these acoustic stimuli, needs to be studied in the future.

#### Multi-peaked neurons versus single-peaked neurons

Cortical neurons with multi-peaked tuning characteristics have been previously observed in several mammalian species. In bats, combination-sensitive neurons with multi-peaked tuning characteristics were found in both primary and secondary cortical areas (Fitzpatrick et al. 1993; Suga 1994) and have been implicated in echolocation behavior. In cats, multi-peaked neurons have been reported in A1 (Abeles and Goldstein 1970, 1972; de Ribaupierre et al. 1972; Oonishi and Katsuki 1965; Phillips and Irvine 1981; Reale and Imig 1980; Sutter 2000; Sutter and Schreiner 1991). In marmosets, some examples of multi-peaked neurons were reported (Aitkin and Park 1993). The rate of occurrence of multi-peaked neurons varied between species and between investigations of the same species, the latter may have resulted from sampling biases between the studies (Sutter and Schreiner 1991). In the present study, the majority of A1 units (~80%) were found to have single-peaked tuning characteristics.

Single- and multi-peaked A1 units share some functional properties. They both can be modulated by distant off-CF inputs that could be facilitatory or inhibitory, depending on the specific frequency and sound level of the off-CF tone. However, these two types of A1 units appear to differ in some fundamental ways. The two-tone facilitation and inhibition observed in single-peaked units are not predictable from their single-tone responses because these units do not respond to off-CF tones when presented alone. More importantly, multi-peaked units exhibited harmonically related two-tone facilitation (Figs. 4 and 5), whereas harmonically related inhibition was found to be closely associated with single-peaked units



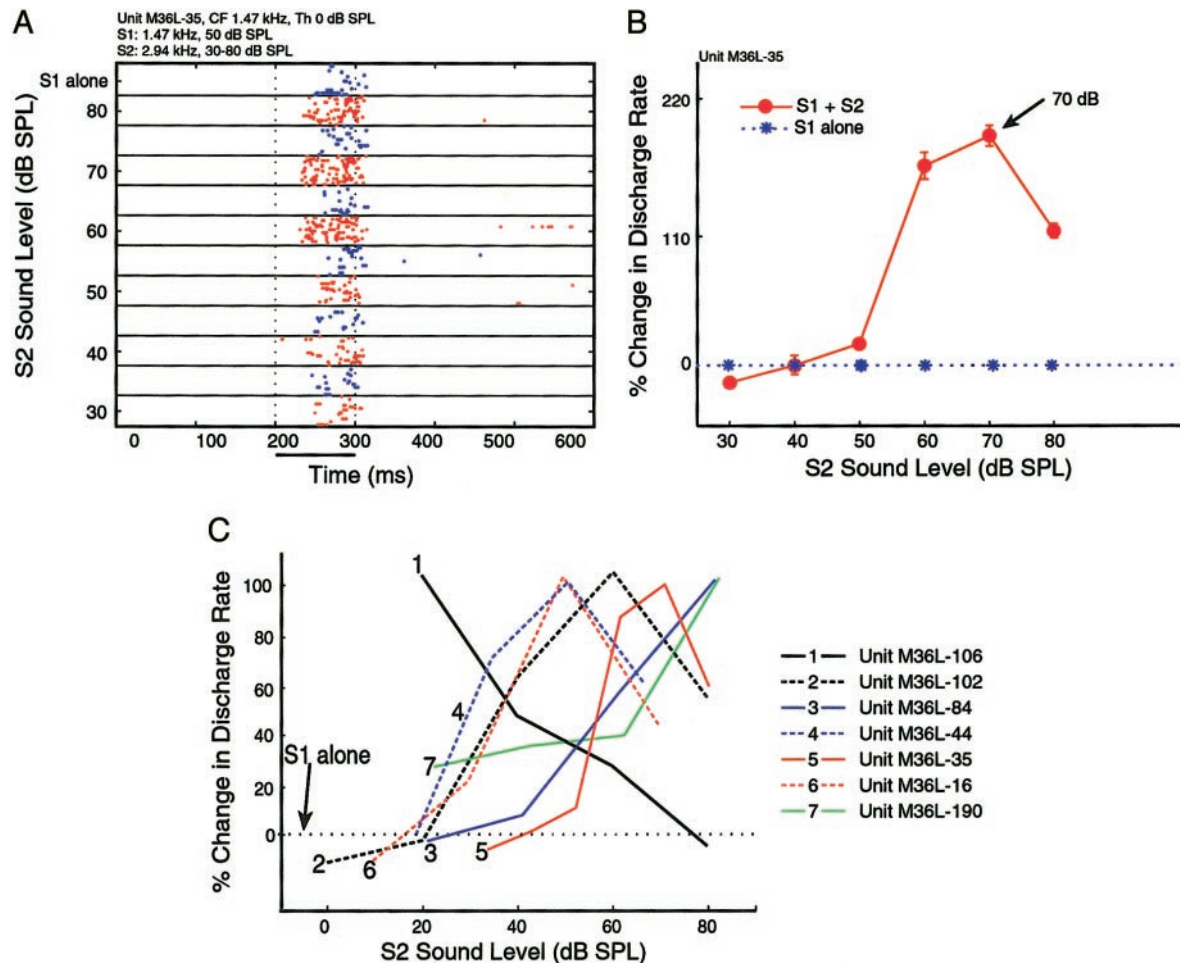


FIG. 13. Sound level dependency of 2-tone facilitation in single-peaked units. *A*: an example showing the effects of varying S2 sound level on 2-tone facilitation (the same unit as shown in Fig. 7, *C* and *D*). Sound level of S1 tone (at unit CF, 1.47 kHz) was kept constant at 50 dB SPL. S2 tone was set to 2\*CF (2.94 kHz) with its sound level varying from 30 to 80 dB SPL (in 10-dB steps). Each alternate block represents a 2-tone condition (red), whereas the other blocks represent a control condition, i.e., CF-tone played alone (blue). *B*: percent change in discharge rate is plotted vs. S2 sound level based on the data shown in *A* (red). Discharge rates of the responses to S1 alone (at CF, 50 dB SPL) are shown by asterisks. The dotted line indicates the average response to the S1 tone played alone. *C*: examples of 2-tone response vs. S2 sound level functions for a small group of single-peaked units. Unit 5 is the same unit shown in *A* and *B*. The horizontal dotted line indicates the average response in the control condition (S1 tone played alone).

(Fig. 10). The anatomical substrates that give rise to the physiological properties in both types of neurons may be similar. The functional differences between these two types of neurons could arise from different distributions of synaptic weights among a neuron's multiple off-CF inputs. In this case, multi-peaked units can be considered as the special cases of single-peaked units. However, it is entirely possible that the multi- and single-peaked units are differently innervated by thalamocortical and/or corticocortical connections.

#### Combination sensitivity and multi-peaked neurons

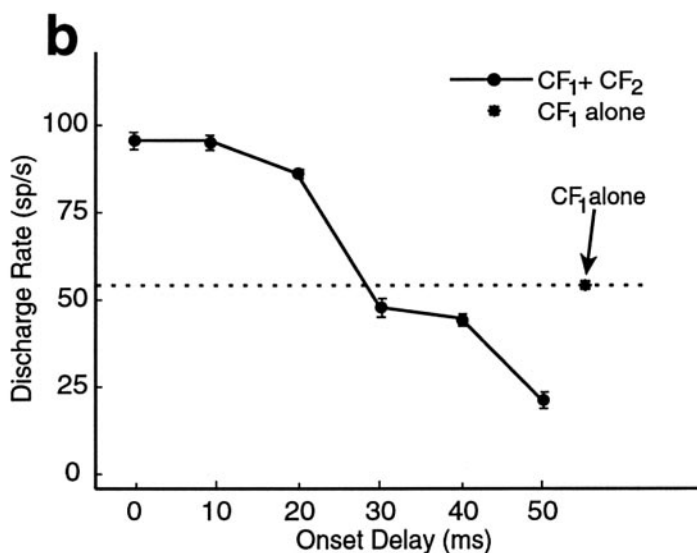
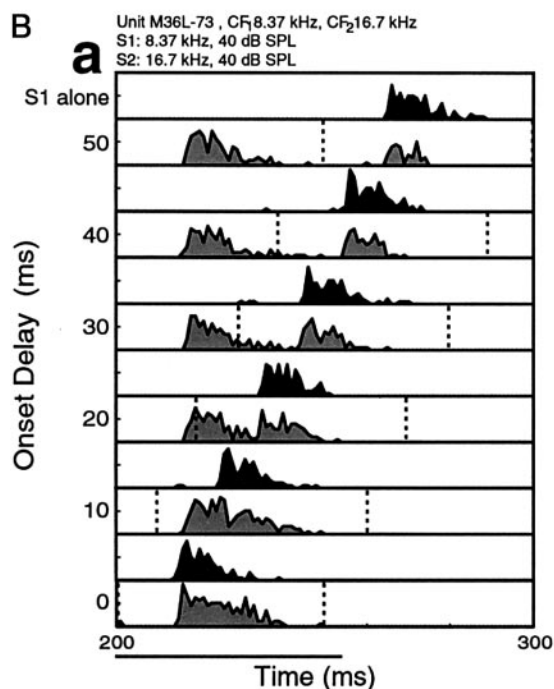
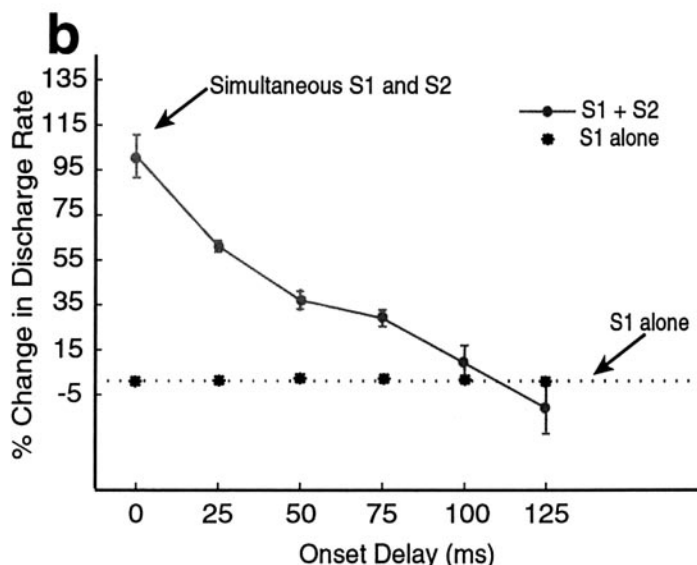
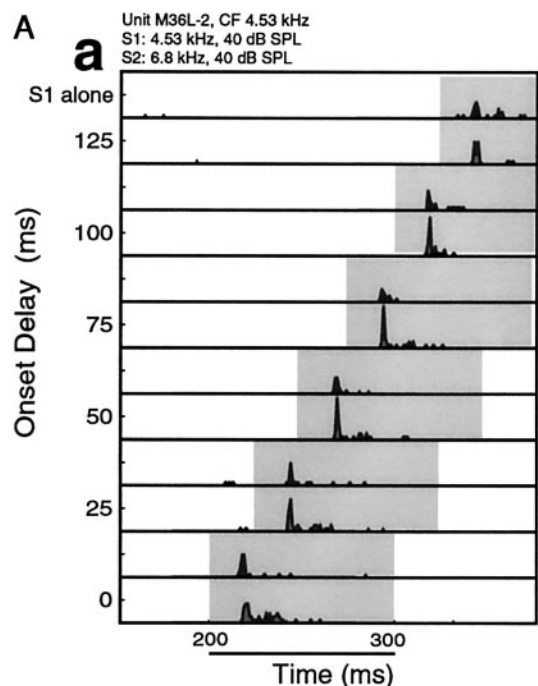
Combination sensitivity has been demonstrated as a neural mechanism for target identification by neurons with multi-peaked tuning characteristics in the echolocating bat (see review by Suga 1994). Using the stimuli consisting of combinations of information-bearing elements in a bat's sonar pulse and echo, combination-sensitive neurons have been found in various structures of the mustached bat's auditory system (IC:

Mittmann and Wenstrup 1995, MGB: Olsen and Suga 1991, A1: Fitzpatrick et al. 1993; Kanwal et al. 1999; other cortical fields: Suga et al. 1978, 1983, 1997). Responses of these neurons were facilitated by combinations of two or more harmonically related constant-frequency or frequency-modulated tones. In bats, most combination sensitive neurons were found in a narrow range of frequencies near harmonics of 30 kHz, the fundamental component in echolocating signals of mustached bats. In many cases, these neurons respond poorly to one tone presented alone but were facilitated by simultaneous presentation of the combinations of tones simulating a sonar pulse and echo at appropriate sound levels. Recent studies suggest that combination-sensitivity may also be involved in the processing of communication sounds in bat auditory cortex (Esser et al. 1997; Kanwal et al. 1999).

The results from our study of A1 neurons in awake marmosets shared some similarities with the observations in A1 of unanesthetized bats (Fitzpatrick et al. 1993; Kanwal et al. 1999). There were, however, some differences between two-

tone responses recorded from the auditory cortex of marmosets and bats. First, temporal response patterns of marmoset A1 neurons were generally different from those in bats. For example, A1 neurons in bats displayed primarily onset responses, whereas both onset and sustained responses were commonly observed in marmosets. Two-tone facilitation could be observed in either of these temporal components in marmoset A1 neurons and was often manifested in the sustained discharges (e.g., Fig. 5A). Second, combination-sensitive neurons in bat A1 responded to the combination of two spectral components with a temporal delay between them (Fitzpatrick et al. 1993;

Kanwal et al. 1999), whereas those in marmosets appeared to prefer nearly simultaneous two-tone presentation (Fig. 14). Moreover, the multi-peaked neurons in marmoset A1 had much lower CFs than those found in bats. As many studies have demonstrated, details of the functional organization of the auditory system should differ from species to species according to species-specific acoustic experience and behavior. Our findings have extended observations in bats to a species that does not use echolocation in its natural behavior and thus support the notion that combination sensitivity is a general phenomenon across species.





### Functional implications of the harmonic structure in multi-peaked neurons

Our everyday auditory experience involves sounds from different sources with overlapping spectral components. Listeners are aided by various fundamental features of complex sounds, such as harmonicity, to group together components of the same source and to separate sounds from other sources (Bregman 1990; Darwin 1997; Stevens and Davis 1938). The process involved in auditory grouping and segregation has been examined in a number of psychophysical studies (Darwin and Hukin 1998; Darwin and Sutherland 1984). A feature common to many aspects of auditory processing is that the perception of acoustic attributes is largely dependent on the context within which a sound is heard. For example, the concurrent presentation of harmonically related frequencies gives a percept of a fused, single, complex tone, whereas delaying the onset of a component in a harmonic series causes it to stand out perceptually (Bregman 1990). Multi-peaked neurons in the auditory cortex could be possible neural substrates subserving such perceptual tasks. Neurons with facilitative interactions between harmonically related peaks are optimally stimulated by specific combinations of spectral elements. Therefore these neurons can function to extract harmonic components embedded in complex sounds as a unitary object. It is also conceivable that such neural mechanisms can facilitate detection of signals in noisy environments by binding spectrotemporal features of an acoustic object.

Both human speech and music contain abundant harmonicity as a context in which other spectrotemporal features are present. For example, the fundamental frequency ("pitch") of a vowel and its harmonics are crucial for the perception of speech, in particular between multiple speakers or in noisy environments (Darwin and Carlyon 1995). Species-specific vocalizations of marmosets are also rich in harmonics (Agamaite and Wang 1997; Epple 1968). The functional properties of A1 neurons revealed here suggest context-dependent mechanisms by which biologically relevant acoustic information can be perceived. One may speculate that the cortical neural circuitry has evolved to accommodate the sensory environments with the prevalence of harmonicity in natural sounds.

### Facilitatory influences from outside the classical receptive fields in single-peaked neurons

The facilitatory responses to two-tone combinations of harmonically related frequencies observed in some single-peaked neurons are similar to the facilitation seen in multi-peaked neurons. When facilitation was observed, the two-tone responses were manifested either in the form of strengthened responses (e.g., sustained discharges near  $1.5 \times \text{CF}$  in Fig. 9B) or the appearance of a new response component (e.g., a new onset response in Fig. 9A). In most cases where the temporal separation of two tones was examined, the facilitatory responses were observed when both tones overlapped in time and the strongest facilitation was achieved when the onset of the tones was nearly simultaneous (Fig. 13). Two-tone facilitation generally became weaker with increasing onset delay (Fig. 13). Some degree of overlap between two tones appeared to be necessary to achieve significant facilitation, a property that was observed for both single-peaked and multi-peaked units (Fig. 13, A and B). These results are different from those reported by Brosch et al. (1999) in anesthetized macaque monkeys, where the maximal facilitatory responses were observed when the onsets of two nonoverlapping tones (duration 100 ms) were separated by  $\sim 120$  ms. Such differences could be due to the effects of anesthetics or different cortical layers where recordings in the two studies were made. In Brosch et al. (1999) study, the results were obtained from areas A1 and CM in macaques, although no obvious differences between these two areas were found in their analyses. It is possible that the facilitation observed in our study and that reported by Brosch et al. (1999) arise from different cortical circuits and mechanisms. Brosch et al. (1999) also reported that maximal two-tone enhancement was more frequently observed when two nonoverlapping tones were separated in frequency by approximately one octave. We did not observe in single-peaked units a strong preference for one-octave separation between two tones in facilitatory modulation (Fig. 10A) but found a clear preference in inhibitory modulation when the second tone was one octave higher or lower in frequency than the first tone (Fig. 10B). For the multi-peaked units we studied, a preference for one-octave separation between two tones in facilitatory modulation was observed (Figs. 4B 6). Brosch et al. (1999) did not indicate whether there were multi-peaked single units in their samples that contained largely multiunit clusters.

FIG. 14. Effects of varying the onset delay on 2-tone responses. *A*, *a*: 2-tone responses (shown in the form of PSTHs) as a function of the onset delay for the single-peaked unit shown in Fig. 7, A and B. The frequencies and sound levels of both S1 (CF, 4.53 kHz) and S2 ( $1.5 \times \text{CF}$ , 6.8 kHz) tones were held constant. The frequency of the S2 tone was near the facilitation peak in this unit (Fig. 7, A and B). The onset delay was varied from 0 to 125 ms (in steps of 25 ms). Each alternate block represents a 2-tone condition, whereas the other blocks represent a control condition (responses to the S1 tone alone). The thick bar below the *x* axis indicates the onset time and duration of the S2 tone. The shaded areas indicate the onset time and duration of the S1 tone (100 ms) played in combination with the S2 tone or alone (in alternating blocks, truncated in the display at the onset time of 100 and 125 ms). *b*: percent change in discharge rate vs. the onset delay for the data shown in *A*. The discharge rates were calculated over the time window indicated by the shaded area in *a* for each stimulus condition. Discharge rates of the responses to S1 alone are shown by asterisks. The dotted line indicates the average response to the S1 tone played alone. *B*, *a*: 2-tone responses as a function of the onset delay recorded from a multi-peaked unit (CF<sub>1</sub>: 8.37 kHz, CF<sub>2</sub>: 16.7 kHz). The frequencies and intensities of both S1 (at CF<sub>1</sub>) and S2 (at CF<sub>2</sub>) tones were held constant. The onset delay was varied from 0 to 50 ms (in steps of 10 ms). Each alternate block represents a 2-tone condition, whereas the other blocks represent a control condition (responses to the CF<sub>1</sub> tone alone). The thick bar below the *x* axis indicates the onset time and duration of the S2 tone. The vertical dotted lines indicate the time window (50 ms in duration, shifted by the onset delay between the 2 tones) over which the 2-tone responses were compared with the 1-tone responses. *b*: discharge rate vs. the onset delay for the data shown in *a*. The discharge rates were calculated over the time window indicated by vertical dotted lines in *a* for each stimulus condition. Note that the 2-tone response was strongest for the simultaneous presentation of CF<sub>1</sub> and CF<sub>2</sub> tones and decreased in strength as the onset delay between the 2 tones was increased. The dotted line indicates the average response in the control condition (S1 tone played alone).

It needs be emphasized that the two-tone facilitation observed single-peaked units is highly dependent on the sound level of the second tone. This issue has not been explored in previous studies of two-tone facilitation in auditory cortex where two tones of equal intensity were usually used (e.g., Brosch et al. 1999). We found that the facilitation versus the S2 sound level functions can be either monotonic or nonmonotonic (Fig. 13B). The sound-level dependence of the two-tone responses implicates that both amplitude and frequency of stimuli distant from CF are crucial parameters in two-tone facilitation. We believe that the two-tone facilitation is likely to reflect the excitation of neurons providing off-CF inputs.

The two-tone facilitation that we observed in single-peaked units differs in its characteristics from that observed in multi-peaked units. Responses of single-peaked units can be facilitated by harmonically related or unrelated stimuli (Fig. 10A), thus giving these units more general spectral integration properties than the multi-peaked units. In fact, the acoustic experience of marmosets includes combinations of both harmonically related and unrelated frequencies, for example, in their vocalizations (Agamaite and Wang 1997; Eppl 1968) and in sounds of their prey and predators. Our results suggest that the spectral organization of primate A1 may include second-order structures that can support spectral integration across a wide range of frequencies. This conclusion is significant because most previous studies of the functional organization of A1 have focused on tonotopic representation (Merzenich et al. 1975; Reale and Imig 1980) and spectral integration within the classical receptive field (Schreiner and Mendelson 1990; Shamma et al. 1993).

#### *Distant inhibition in single-peaked neurons*

One of the most important properties of cortical neurons is the presence of inhibition flanking the excitatory receptive field. The flanking inhibition observed at central auditory nuclei is different from the two-tone suppression described in the auditory nerve (Sachs and Kiang 1968) because two-tone suppression can only occur for simultaneous stimuli. Several studies have provided evidence of two-tone inhibition at various stages of the auditory pathway including auditory cortex (Brosch and Schreiner 1997; Calford and Semple 1995; Ehret and Merzenich 1988; Kanwal et al. 1999; Phillips 1988; Riquimaroux 1994; Shamma et al. 1993; Suga 1965a,b; Sutter et al. 1999). A main finding of the present study is the harmonically related distant inhibition in single-peaked units. Such inhibitory modulations were produced by tones with frequencies far away from the CF of a unit and were distinctly different from the well-documented flanking inhibitory regions near CF. Distant inhibitory influences in A1 have been observed in the auditory cortex of other nonprimate species (e.g., bat: Kanwal et al. 1999; cat: Sutter et al. 1999). These findings indicate that an A1 neuron receives inhibitory inputs from a wide range of frequencies. As our findings showed, the distant inhibitions were often harmonically related to the CF of a single-peaked unit (Fig. 10B). In contrast, this harmonic structure was not prominently present in facilitatory modulations observed in single-peaked units (Fig. 10A). The frequency specificity of the distant inhibition in the single-peaked units suggests that the distant off-CF inhibition may have a different functional role than the distant off-CF facilitation. It further

suggests that single-peaked neurons, which represent the majority of A1 neurons in marmosets, may process harmonically related spectral components in a different manner than multi-peaked A1 neurons.

The harmonically related inhibition described here may provide explanations for certain perceptual phenomena. Just as harmonics are useful in assembling components of a complex acoustic object into one entity, they can also introduce confusion regarding the identification of the fundamental frequency. The strong inhibitory influences by harmonically related frequencies might enhance the perception and identification of the fundamental frequency. Together, the facilitatory and inhibitory modulation in the multi-peaked and single-peaked neurons can constitute the fundamental building blocks for cortical representations of communication sounds.

#### *Potential neural mechanisms underlying two-tone interactions in A1*

It has long been known that there exist extensive long-range horizontal connections within the primary sensory cortex including A1 formed by the axons of pyramidal cells. These horizontal connections, found in the supragranular layers of cat A1, extend as long as a few millimeters and span up to two octaves in CFs (Kadia et al. 1999; Matsubara and Phillips 1988; Ojima et al. 1991; Reale et al. 1983; Wallace et al. 1989; Winer 1992). They may possibly provide distant off-CF inputs to A1 neurons that underlie harmonically related facilitation and inhibition observed in our study. Although long-range horizontal connections have not yet been directly demonstrated in the auditory cortex of the common marmoset, their existence in other primate and mammalian species suggests strongly that this anatomic feature is preserved in the sensory cortex across many species. It has been suggested that spatial integration over a large visual space in the primary visual cortex may be mediated by long-range horizontal connections (Gilbert 1998; Gilbert and Wiesel 1979; McGuire et al. 1991). In two-tone responses of A1 neurons, the sound level of the off-CF tone often determines if facilitation or inhibition is evoked (Figs. 11–13). This result is consistent with the observations in primary visual cortex that changes in stimulus intensity could alter the relative contribution of excitatory and inhibitory responses evoked at a single cortical site (Hirsch and Gilbert 1991; Kapadia et al. 2000; Weliky et al. 1995). Because horizontal connections originated from pyramidal cells are primarily excitatory (Gilbert and Wiesel 1979; Martin and Whitteridge 1984) and innervate both excitatory and inhibitory, GABAergic neurons (Kisvarday et al. 1986; McGuire et al. 1991), they may contribute to both the distant facilitation and inhibition as reported in the present and previous studies using two-tone stimuli.

Because response properties observed in the auditory cortex represent cumulative effects of processing at each location along the auditory pathway, it is difficult to assess the contributions of cortical and subcortical neural circuits solely on the basis of cortical responses. Enhanced neural responses by two tones have been observed in some subcortical nuclei, for example, in ventral cochlear nucleus (VCN) of guinea pigs (Jiang et al. 1996; Winter and Palmer 1995), lateral lemniscus, IC and MGB in bats (Portfors and Wenstrup 1999, 2001; Wenstrup 1999). However, such enhancement appears to be less promi-

ment than that observed in A1. Another potential source of off-CF inputs to A1 neurons is via thalamocortical connections (DeVenecia and McMullen 1994; Hashikawa et al. 1995), although the extent of such inputs across tonotopic axis is more limited than that of long-range horizontal connections observed in A1.

Finally, one may speculate what could be the functional significance of the harmonic structure associated with inhibition in single-peaked neurons and facilitation in multi-peaked neurons. We have suggested that the harmonically related facilitation could facilitate the detection of harmonic components embedded in complex sounds and that the harmonically related inhibition could serve as a mechanism to remove unwanted harmonic artifacts in natural environment. It is also conceivable that, by combining activities of these two distinct types of responses, the auditory cortex could determine whether a sound is harmonic, a function that is important for a wide range of acoustic perception.

We thank Dr. David Ryugo for providing support during this work, Dr. Ross Snider for technical assistance in writing data acquisition and stimulus generation software, and A. Pistorio for animal care, for graphic assistance, and for proofreading the manuscript. Drs. Edward Bartlett and John Doucet provided helpful comments on the manuscript. We also thank two anonymous reviewers for constructive suggestions.

This work was supported by National Institute on Deafness and Other Communication Disorders Grant DC-03180 and by a Presidential Early Career Award for Scientists and Engineers (X. Wang).

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