Excitatory and inhibitory intensity tuning in auditory cortex: evidence for multiple inhibitory mechanisms.

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Abstract

The intensity tuning of excitatory and suppressive domain frequency response areas (FRAs) was investigated in 230 cat primary auditory cortical (A1) and 92 posterior auditory field (PAF) neurons. Suppressive domains were explored using simultaneous two-tone stimulation with one tone at the best excitatory frequency (BEF). The intensity-tuning of excitatory and suppressive domains were negatively correlated, supporting the hypothesis that inhibitory sidebands are related to excitatory domain intensity tuning. To further test this hypothesis, we compared the slopes of the edges of suppressive bands to the intensity tuning of excitatory domains. Edges of suppressive bands next to excitatory domains had slopes significantly more slanted towards the excitatory area in neurons with intensity tuned excitatory domains. This relationship was not observed for suppressive band edges not next to the excitatory domain (e.g., the lower edge of lower suppressive bands). This indicates that intensity tuning ultimately observed in the excitatory domain results from overlapping excitatory and inhibitory inputs. In combination with results using forward masking (Calford & Semple 1995), our results suggest that there are separate early and late sources of inhibition contributing to cortical frequency response areas, and only the early-stage inhibition contributes to excitatory domain intensity-tuning.

Key words – auditory cortex, non-monotonic, inhibition, receptive field, posterior auditory field
INTRODUCTION

Neurons that are tuned for sound intensity (also called nonmonotonic neurons) are common throughout the central auditory system. Intensity tuning is interesting from both a functional and mechanistic perspective. Intensity tuning is potentially important for loudness encoding (Phillips 1993; Phillips & Carr 1998), analyzing complex sound spectra (Sutter & Schreiner 1995; Phillips 1993), spectral analysis in the presence of noise (Phillips 1987; 1990), and envelope sensitivity (Phillips 1988; Heil & Irvine 1998; Heil 1998; Phillips et al. 1995), and must be accounted for in any population code of auditory signal processing (Semple & Kitzes 1993b; Phillips et al. 1994). The mechanisms underlying intensity tuning are intriguing because they must rely on neural inhibition. Inhibition from the central nervous system is required because all auditory nerve fibers, the input to the central auditory system, have monotonically increasing response vs. intensity functions. Therefore, to reduce activity preferentially at high intensities requires inhibition.

Intensity-tuned neurons can be found in most auditory brain areas, including the cochlear nucleus (CN) (Greenwood & Maruyama 1965; Young & Brownell 1976), inferior colliculus (IC) (Aitkin 1991; Kuwabara & Suga 1993), medial geniculate body (MGB) (Aitkin & Webster 1972; Rouiller et al. 1983; Rodrigues-Dagaeff et al. 1989) and cortex (AC) (Erulker et al. 1956; Evans & Whitfield 1964; Phillips & Irvine 1981; Barone et al. 1996). Ionophoretic injection of GABAergic and glycinergic receptor agonists and antagonists indicate that intensity-tuning is constructed de novo in many auditory areas (e.g., CN: Caspary et al. 1979; Evans & Zhao 1993; Davis & Young 2000; SOC: Grothe 1994; Zheng & Hall 2000; IC: Faingold et al. 1991; Vater et al. 1992; Yang et al. 1992; Pollak & Park 1993; Fuzessery & Hall 1996; Chen & Jen 2000; Hall 1999; MGB: Suga et al. 1997; AC: Wang et al. 2000). In primary auditory cortex (A1), $\frac{1}{4}$ to $\frac{1}{2}$ of the neurons are intensity tuned (e.g., Phillips & Irvine 1981; Sutter & Schreiner 1995; Clarey et al. 1994; Imig et al. 1990), depending on the definition of intensity tuning and the experimental methods used (Phillips 1988; Heil 1997). Although intensity tuned neurons
can be found throughout A1, two areas in A1 have a concentration of intensity tuned cells resulting in a topographic map of intensity (Sutter & Schreiner 1995; Heil et al. 1994; Suga & Manabe 1982).

The reduction in responsiveness at high intensities in intensity-tuned neurons may be produced by the overlap of non intensity-tuned excitatory and inhibitory inputs. Greenwood and Maruyama (1965) concluded on the basis of firing patterns of cochlear nucleus neurons that many stimuli evoke both excitatory and inhibitory processes with the net effect on firing determined by the relative timing and strength of the excitation and inhibition. Phillips (1988) found evidence that excitatory domain (ED-) intensity-tuning of cortical neurons was related to lateral inhibitory processes, and discussed the possibility that ED-intensity-tuning emerges from the overlap of an inhibitory input with a non intensity-tuned excitatory input at high intensities. The schematic in Figure 1A illustrates how this might occur. The frequency tuning of level one neurons that provide input are sufficiently broad so that inhibitory and excitatory inputs overlap in the level two neuron. And because the inhibitory synapses are as strong as the excitatory synapses, the inhibition cancels the excitation in the region of overlap, with the net result that the excitatory domain is intensity-tuned and the inhibitory domains are not intensity-tuned. Figure 1B illustrates a similar scenario, except that the inhibitory synapses are weaker than the excitatory synapses. As a result, level two neurons have a non intensity-tuned excitatory domain flanked by intensity-tuned inhibitory domains that slant away from it. In the third scenario (Figure 1C) the inputs are tuned sufficiently narrow so that inhibitory and excitatory inputs do not overlap. As a result, both the excitatory and inhibitory domains are not intensity-tuned in level two neurons. Note that it would not be possible to derive intensity tuning for both the excitatory and inhibitory domains. Thus, this model predicts that the shapes and intensity tuning of excitatory and inhibitory domains will be correlated in certain ways. An experimental goal of this paper is to test this prediction.

Measuring inhibitory/suppressive response areas of cortical neurons in anesthetized preparations is complicated by low spontaneous firing rates. The only study examining the correlation in the inten-
sity-tuning of inhibitory and excitatory domains in A1 neurons is Calford and Semple (1995). They used a forward masking stimulus paradigm, in which an initial tone is followed by a second tone in the neuron’s excitatory domain, to reveal persistent inhibition. It was observed that excitatory and inhibitory domain properties were correlated, but not in the manner predicted by the model described above. For example, neurons with intensity-tuned excitatory domains tended to have forward masked inhibitory domains that were also intensity-tuned and that did not account for the excitatory tuning. The authors hypothesized that the inhibition responsible for creating intensity-tuned excitatory domains was distinct from forward masking inhibition. Another goal of this paper is to expand on this idea, and propose that ED-intensity-tuned cortical neurons display at least two types of inhibition: i) a short latency, short acting inhibition that abolishes responses at high intensities and thereby carves out intensity tuned excitatory domains, and ii) a longer latency, longer lasting lateral inhibition derived from local clusters of intensity-tuned cells which does not contribute to ED-intensity tuning.

Short latency inhibition relative to excitation may be necessary to produce ED-intensity tuning at higher levels of the auditory system, where the responses are primarily phasic, rather than sustained. Most cat A1 neurons produce only a short latency phasic onset response where all spikes fall in a narrow time probability window (~5-10 msec). Furthermore, for many ED-intensity-tuned A1 neurons, this onset response is completely abolished by inhibition at high intensities (e.g., Phillips et al. 1985; Phillips et al. 1995). In order for inhibitory postsynaptic potentials (ipsps) to create the complete cessation of short latency phasic onset responses at high intensities, they must arrive nearly simultaneously with excitatory postsynaptic potentials (epsps). Otherwise, part of the onset response could occur before the inhibition can exert an effect. This stands in contrast to earlier stations of the auditory system where intensity-tuning can result from inhibition later in a sustained responses (e.g., Shofner & Young 1985; Spirou et al. 1999; Rhode et al. 1983; Rhode & Smith 1986; Rhode & Kettner 1987; Ramachandran et al. 1999). Thus, in the model of Figure 1, level one neurons are connected to level two
neurons via a fast, monosynaptic inhibitory pathway and a delayed, disynaptic excitatory pathway. The inhibition that underlies ED-intensity-tuning in A1 neurons does not necessarily reside in A1, but may be derived from any areas in the auditory pathway. But wherever the source of the inhibition is located, it must have a short latency compared to excitatory inputs in the same region. At the same time, one cannot automatically assume that ED-intensity tuning in A1 neurons is inherited from ED-intensity-tuned neurons at earlier stages of the pathway.

The posterior auditory field (PAF, also called ‘field P’) is a cortical area where roughly 80% of the neurons have intensity-tuned excitatory domains (Kitzes & Hollrigel 1996; Phillips & Orman 1984; Heil & Irvine 1998). Despite the prevalence of ED-intensity tuning in PAF, measurements of inhibitory/suppressive domains and their relationship to ED-intensity tuning in PAF have been lacking. Although PAF neurons have different spectral and temporal properties than A1 neurons (e.g., Loftus & Sutter 2001b), Phillips et al. (1995) hypothesized that mechanisms responsible for ED-intensity tuning in PAF neurons may be similar to those for ED-intensity tuning in A1 neurons. If this is correct, then the correlations between the suppressive and excitatory domain shapes that we find in A1 should also be observed for PAF.

In the present paper, we measure excitatory and suppressive domains of A1 and PAF single neurons. Simultaneous two tone stimulation is used in order to reveal the effects of short latency inhibition. Quantitative measures of intensity tuning and other aspects of response domain shape are derived. Correlations between the excitatory and suppressive domains are examined and compared to the model predictions, and the model is expanded to account for both simultaneous and forward masking data.

MATERIALS AND METHODS

Surgical Preparation

We recorded single neurons from A1 in 3 left, and 23 right hemispheres of 25 young adult cats, and from PAF in 6 left and 2 right hemispheres of 8 young adult cats. Surgical preparation, stimulus de-
livery and recording procedures for A1 are the same as those from a previous study (Sutter & Schreiner 1991), with exceptions noted below. Stimulus delivery and recording procedures for PAF are the same as those in (Loftus & Sutter 2001b), with exceptions noted below.

Briefly, anesthesia was induced with an i.m. injection of ketamine hydrochloride (10 mg/kg) and acetylpromazine maleate (0.28 mg/kg). After venous cannulation, an initial dose of sodium pentobarbital (30 mg/kg) was administered. Animals were maintained at a surgical level of anesthesia with a continuous infusion of sodium pentobarbital (2 mg/kg/hour) and if necessary, with supplementary i.v. injections. Lactated Ringer's solution was injected through a separate catheter for a total fluid volume of 3.5-4.0 ml/kg/hr. The cats were also given dexamethasone sodium phosphate (0.14 mg/kg, IM) to prevent brain edema, and atropine sulfate (1 mg, IM) to reduce salivation. The temperature of the animals was monitored with a rectal temperature probe and maintained between 37.5 and 38.0°C by means of a heated water blanket with feedback control.

Three-point head fixation was achieved with palatal-orbital restraint, leaving the external meati unobstructed. The temporal muscle was retracted and the lateral cortex exposed by craniotomy. The dura overlying the middle and/or posterior ectosylvian gyrus was removed, the cortex was covered with silicone oil, and a video image of the surface vasculature was taken to record the electrode penetration sites. If brain pulsation interfered with stable single unit recording, a semi-closed system was used consisting of a wire mesh placed over the craniotomy. The space between the grid and cortex was filled with a 1% solution of clear agarose. The agarose-filled grid/chamber diminished pulsation of the cortex and provided a fairly unobstructed view of identifiable locations across the exposed cortical surface.

To confirm electrode locations, near the end of some experiments (72-120 hours of recording) penetrations were marked with 2 or 3 electrolytic lesions (~10 µa DC; electrode negative; ~10 s). The cats were transcardially perfused with physiological saline followed by 4% paraformaldehyde. The brains were blocked and stored in 10% sucrose. Frozen sections were cut in the horizontal plane at a
thickness of 50-60 µm, counterstained with cresyl violet, and examined under a light microscope. A1 and PAF borders were defined from the established tonotopic borders (Reale & Imig 1980; Merzenich et al. 1975). Additional confirmation that recordings were in PAF was provided by retrograde tracer experiments in two animals. In both cases, several tracer injections were made along the dorsal-ventral extent of putative PAF, as defined by the physiological criteria of the present study. The resulting pattern of labeling within the medial geniculate body (MGB) matched the expected distribution of thalamic projections to PAF (Morel & Imig 1987; Rodrigues-Dagaeff et al. 1989).

**Stimulus Generation and Delivery**

Experiments were conducted in double-walled sound-shielded rooms (IAC). Stimuli were generated by a microprocessor (TMS32010 or TDT; 16 bit D/A converter at 120 kHz; low-pass filter of 96 dB/octave at 15, 35 or 50 kHz). A pair of passive attenuators provided attenuation.

For A1 neurons, sounds were delivered with insert speakers. We used calibrated headphones (STAX 54) enclosed in small chambers that were connected to sound delivery tubes sealed into the acoustic meati. This sound delivery system was calibrated with a sound level meter (Brüel & Kjaer) and distortions were measured either with waveform analyzers or a computer acquisition system. The frequency response of the system was essentially flat up to 14 kHz and did not have major resonances deviating more than +/- 6 dB from the average level. Above 14 kHz, the output rolled off at a rate of 10 dB/octave. Because our calibration ignores potential influences of the outer ear, measurements of the slopes of frequency tuning curves might be affected, particularly at high frequencies. Slope measurements were adjusted for the transfer function rolloff, but this does not include the influences of notches in the head-related transfer function. However, since we sampled neurons with a wide range of best frequencies, this likely changed the variance of inverse slope measures and less likely the mean. Harmonic distortion was better than 55 dB below the primary.

For PAF, auditory stimuli were presented ‘near field’ (speaker distance to the center of the head
3.0 ft) via calibrated speakers: a Radio Shack Optimus Pro-7AV and a Radio Shack dual radial horn tweeter (cat. # 40-1377) with a crossover circuit at 7kHz. A Radio Shack MPA 200 amplifier drove the speakers. Speakers were placed at +/- 90 degrees azimuth and 0 degrees elevation relative to the animal and oriented directly toward the pinna contralateral to the recorded hemisphere. The sound system was calibrated with a sound level meter (Brüel & Kjaer type 2231) with a probe microphone positioned near the pinna. The frequency response of the system was essentially flat from 0.5 to 40 kHz except for two notches of less than 14 dB (peak to peak) centered at 1.2Khz and 2.8kHz; otherwise, major resonances deviated less than +/- 6 dB from the average level. Above 40kHz, the output rolled off at a rate of 37 dB/octave. Harmonic distortion was less than 60 dB below the primary.

Pure tone bursts were 50 ms in duration with a 3 ms rise/fall time. To assess suppressive domains, two-tone bursts were constructed digitally by combining two simultaneous pure tones with the same shaping as the pure tone bursts. The interstimulus interval was 400-1200 ms for pseudo-randomly presented pure tones and 600-2000 ms for pseudo-randomly presented tone-pairs.

**Recording Procedure**

Parylene-coated tungsten microelectrodes (Microprobe Inc.) with impedances of 1-10 MOhm at 1 kHz were introduced into the auditory cortex with a hydraulic microdrive. For A1, all penetrations were approximately orthogonal to the brain surface; For PAF, penetrations were made into the caudal bank of the posterior ectosylvian sulcus, to depths up to 3300 microns, and located at various dorsoventral positions and distances from the sulcus (200 - 1200 microns). Histological verification from several animals indicated that lesioned recording sites from A1 were from cortical layers 3 and 4; from PAF the recording sites were more distributed throughout the cortical layers. Neuronal activity of single units was amplified, band pass filtered, and monitored on an oscilloscope and an audio monitor. During early experiments, action potentials from individual neurons were isolated from background noise with a window discriminator (BAK Electronics) and the time of each action potential was saved to disk. Dur-
ing later experiments, online single unit isolation was performed with software, and spike waveforms were saved to disk to allow for additional offline discrimination after the experiments.

**Single-tone Frequency Response Areas:**

Frequency response areas (FRAs) were obtained for each recorded unit. A description of these procedures can be found in (Sutter et al. 1999), and are very similar to those of (Evans 1974). Briefly, we presented 675 tone bursts in a pseudo-random sequence of different frequency-intensity combinations selected from 15 intensities and 45 frequencies. The intensities were spaced 5 dB apart for a total of 75 dB presented range. The frequency range covered by 45 steps ranged between 2.0 and 5.6 octaves, depending on the estimated width of the frequency tuning curve. Typically we used a three-octave range which provided 0.067-octave resolution between frequency samples. In later experiments the number and range of the tested frequencies could be more flexibly varied to allow the investigators to obtain a high resolution response area covering the full range of the neuron’s response.

Because of the time constraints of single unit recording, we characterized FRAs based on as few stimulus repetitions as possible. If a response was evoked by more than approximately 50% of the stimuli inside of each excitatory band, the curve was deemed well defined. If after one presentation per frequency/intensity combination, the resulting FRA was not well-defined, the process was repeated with the same 675 stimuli and the resulting evoked activity was added to the first. If necessary, the FRA recording procedure was repeated up to five times. This method has provided statistically reliable characterizations of cells based on repeated measure controls (see Table 3 of Sutter and Schreiner 1991).

**Two-Tone frequency response areas**

For most single-neurons recorded in cat auditory cortex under barbiturate anesthesia, spontaneous activity is very low, and does not provide sufficient activity to judge background suppression by a stimulus. Therefore, we used a two-tone simultaneous masking paradigm to measure response suppression. For two-tone FRAs, 675 different tone-pairs were presented. For each tone-pair, one component
(the “BEF tone” or “probe-tone”) was at the cell's best excitatory frequency (BEF) with energy just above response threshold. The BEF was determined from a single tone FRA. The second component (the "variable tone" or “masker tone”) had a frequency and intensity chosen using the same pseudo-random procedure as described for the single tone FRA. The purpose of this element was to determine which frequency/intensity ranges suppressed the activity associated with the fixed BEF tone. If the response to the fixed BEF tone was not reliable (e.g., the mean of the BEF tone activity, in spikes per presentation, was less than the standard deviation or the probability of response less than 0.25), the procedure was repeated with the same 675 tone-pairs. The resulting evoked activity of multiple presentations was then added. Because we presented over 675 stimuli that contained the fixed BEF tone, habituation and/or adaptation sometimes caused the response to decrease over time. In those cases, we repeated the two-tone FRA several times.

Suppressive bands were identified in the two tone FRAs using the methods of Sutter et al 1999. A suppressive band is defined as a contiguous frequency/intensity space on the two-tone FRA where fixed BEF tone activity was reduced by 50% or more by the variable tone. Lower suppressive bands by definition were on the low frequency side of the excitatory domain, and upper bands were on the high frequency side. If there were multiple lower or upper suppressive bands (Sutter et al 1999), only the lower and upper bands closest to the excitatory domain were used. After each band was identified, the best inhibitory frequency (BIF) and threshold was determined for each suppressive sideband.

**Determination of Strength of Excitatory Domain Intensity Tuning**

We used the monotonicity ratio to determine the strength of ED-intensity tuning (e.g., Sutter & Schreiner 1995). The excitatory monotonicity ratio (eMR) is based on the response vs. intensity function near the unit’s characteristic frequency (CF, the most sensitive frequency, also in this paper called the best excitatory frequency, BEF). Response vs. intensity functions for single tone excitatory bands (Fig. 2 A-C) were created as in (Sutter & Schreiner 1995). At each intensity level, the number of action
potentials from a 1/4 octave bin around the unit’s BEF and a 15 dB wide intensity bin was summed. One-quarter octave usually comprised 4 different frequencies and 15 dB usually covered three levels of intensity. This provided a minimum of 12 different stimulus presentations per data point in the response vs. intensity functions. Only units that were recorded from a minimum of 45 dB above threshold were used to analyze eMR. The eMR is the number of spikes elicited at the highest intensity divided by the number of spikes at the maximum of the response vs. intensity function.

\[
eMR = \frac{\text{Spikes}_{\text{highest_intensity}}}{\text{Spikes}_{\text{maximum}}} \quad \text{(equation 1)}
\]

Therefore, a cell that fired maximally at the highest tested intensity had an eMR of 1 (Fig. 2A); a cell that was completely inhibited at the highest intensities had an eMR of 0. (Similar to Fig. 2C).

**Determination of Inhibitory Strength and Intensity Tuning**

Response vs. intensity functions (Fig. 2 D-F) were also created for suppressive bands from the two-tone FRA. At each level of the variable component of the two-tone stimulus, the number of action potentials from a 1/4 octave bin around each band's BIF and a 15 dB wide intensity bin were summed. We derived two metrics from the suppressive/inhibitory domain response vs. intensity function: the inhibitory strength ratio (iSR) and the inhibitory monotonicity ratio (iMR).

The iSR is 1 minus the ratio between the number of spikes elicited at the highest intensity of the variable component of the two tone stimulus and the number of spikes at the maximum of the suppressive/inhibitory response versus intensity function:

\[
iSR = 1 - \left( \frac{\text{Spikes}_{\text{highest_intensity}}}{\text{Spikes}_{\text{maximum}}} \right) \quad \text{(equation 2)}
\]

Therefore, units that were strongly inhibited at high intensities would have iSR values near 1 (Fig. 2D) and neurons with weak inhibition at higher intensities would have iSR values near 0 (Fig. 2F).

The iMR is more closely analogous to the eMR measure because it is the amount of suppression at the highest intensity divided by the maximal suppression in the suppressive/inhibitory domain response vs. intensity function:
\[
\text{iMR} = \frac{(\text{Spikes}_{\text{probe}} - \text{Spikes}_{\text{highest\_intensity}})}{(\text{Spikes}_{\text{probe}} - \text{Spikes}_{\text{minimum}})} \quad (\text{equation 3})
\]

\text{Spikes}_{\text{probe}} \text{ is the response to the probe tone alone in units of spikes per presentation (dotted lines in Fig. 2 D-F). This is estimated from the 90 presentations to the lowest two ‘masker’ intensities used in the two-tone FRA.} \text{ Spikes}_{\text{highest\_intensity}} \text{ is as in Equation (2) and Spikes}_{\text{minimum}} \text{ is the spikes per presentation at the minimum of the suppressive response vs. intensity function, where suppression is maximal. Suppression is estimated by subtracting the two tone response from Spikes}_{\text{probe}}. \text{ Suppressive bands with monotonically decreasing firing rate (and therefore monotonically increasing inhibitory strength) will have iMR values near 1 (Fig. 2 D), and bands with intensity tuned suppressive domains will have values closer to 0 (Fig. 2F). In cases where the highest intensity response is greater than the probe response, the iMR is clipped to zero rather than allowing it to take on negative values (e.g., Fig. 2F). Only units that were recorded from a minimum of 40 dB above suppression threshold were used to analyze iSR and iMR.}

\text{To directly contrast these two measures, the } \text{iSR reflects the magnitude of the inhibition at the highest tested intensity, regardless of whether the suppressive-domain response vs. intensity function is intensity-tuned; on the other hand, the iMR is sensitive to the intensity-tuning of the suppressive domain response vs. intensity function, but is normalized for the strength of inhibition at the highest intensity.} \text{ For example, if the response vs. intensity function in Fig. 2D reached a minimum of 0.6 spikes per presentation rather than 0 spikes per presentation, the iSR would be reduced from 1 to ~ 0.5, but the iMR would remain unchanged at 1. Conversely, if the minimum of the suppressive response vs. intensity function in Fig. 2E had reached 0 at +20 dB, thereby forming a deeper trough, the ISR would remain unchanged at 0.68 but the iMR would get slightly smaller (from 0.83 to 0.72).}

\text{The model of Fig. 1A predicts that both absolute level of inhibition (red lines in Fig. 1A and B) and intensity tuning are important contributors to cortical responses. There is no reason, therefore to suspect that one measure is better than the other } a \text{ priori. Accordingly, for simplicity we will only re-}
port results on iMR since it is the most analogous to the eMR measure, except when iMR and iSR differences are substantial enough to influence interpretations.

RESULTS

Descriptive statistics of suppressive and excitatory domain intensity tuning

While the properties of excitatory domain (ED) intensity tuning in A1 and PAF have been reported by several investigators, the properties of inhibitory domain intensity tuning have yet to be reported quantitatively. In this section we will report the descriptive statistics of ED and inhibitory domain intensity tuning in A1 and PAF, and compare the results for the two cortical areas.

A plurality of A1 cells had monotonically increasing response vs. intensity functions, whereas the majority of PAF cells were intensity tuned for BEF tones. Strongly intensity tuned excitatory domains [excitatory monotonicity ratio (eMR) <= 0.5] were observed in 33.0% (76/230) of the recorded A1 cells, intermediately intensity-tuned excitatory domains (0.5 < eMR < 0.8, Sutter and Schreiner, 1995) occurred in 29.1% (67/230), and monotonically increasing response vs. intensity functions (eMR >= 0.8) were observed in 37.8% (87/230) of A1 neurons (Table 1). The distribution was highly non-normal with a median eMR of 0.685 (Fig. 3). PAF had a higher incidence of ED-intensity tuning with 58.7% (54/92), 23.9% (22/92) and 17.4% (16/92) of PAF neurons having strongly, intermediately, and un-tuned excitatory domains, respectively. The median eMR of the relatively flat distribution was 0.44 (Fig. 3). The differences between A1 and PAF in strongly, intermediately and un-tuned cells was significant ($X^2$, $p < 0.0001$), as was the differences in median eMR (Mann-Whitney, $p < 0.0001$).

For suppressive bands, the distributions of intensity tuning and strength measures are more bimodally distributed with an extremely high proportion of suppressive domains that were not tuned for intensity with inhibitory strength ratios (iSRs) and inhibitory monotonicity ratios (iMRs) near 1 (Fig. 3). A summary of the intensity tuning of suppressive bands abutting the excitatory domain can be seen in
Table 1. The median iMR and iSR for lower and upper bands always indicated stronger inhibition at higher intensities in PAF than A1; however the differences between A1 and PAF were small and never reached significance except for the upper band iMR. We also looked at whether the upper band was more tuned than the lower band; once again, differences in the median did not reach significance, although for A1 iMR, A1 iSR, PAF iMR, and PAF iSR, median values were all higher for upper than lower bands. The strong bimodal tendency for these metrics around values of 1 and 0 probably interfered with making statistical comparisons between A1 and PAF and upper and lower bands.

The ED-intensity tuning of a given neuron may have impacted our ability to measure its suppressive domain tuning. If a neuron with strong ED-intensity tuning responded weakly or habituated to repeated presentations of the BEF tone as a result of heightened inhibition, then it might not be possible to collect a two tone FRA for that neuron. This is supported by Table 2, which shows that the subset of neurons for which a two tone FRA could not be derived had stronger ED intensity tuning than the rest of the sample. This was noticeable for PAF, where the median monotonicity ratio was significantly higher for the neurons with two-tone FRAs than for those without (0.51 vs. 0.36, p < 0.05, Mann-Whitney test). In A1, the difference was not significant, consistent with less habituation in A1.

In summary, these data indicate that ED-intensity tuning is stronger in PAF than A1. There may also be overall differences in suppressive domain tuning between A1 and PAF that could be related to differences in the incidence of ED intensity tuning in these two fields. The ability to detect this is weakened by a bias against recording two-tone FRAs from strongly inhibited PAF neurons. We now consider neurons for which both two-tone and single-tone FRAS were obtained (to mitigate this sampling bias effect), and ask whether the tuning of the excitatory and suppressive domains are correlated.

**Relationship of excitatory domain (ED-) intensity tuning to suppressive domain intensity tuning in A1**

The model of Fig. 1A and 1B predicts an inverse relationship between excitatory and suppressive domain intensity tuning; i.e., neurons with intensity-tuned excitatory domains should have non-
intensity-tuned suppressive domains and vice versa. A Spearman rank test demonstrated this relationship in A1 for both lower and upper suppressive bands (p< .05, Table 3).

Although the ρ values for all the Spearman tests were negative, indicating an inverse relationship, a linear trend was difficult to determine because of the non-uniform distribution of eMR and iMR values (Fig. 4). One of the most striking aspects of Fig. 4 was the paucity of cells with both intensity-tuned suppressive and excitatory domains. To better view this effect (in light of the many overlapping points in the scatter plot) we assigned each neuron to one of four quadrants and made a bubble histogram. For simplicity, we will describe the method only for iMR_lower vs. eMR (Figs. 4 and 5A, left) (although the same method was used for iMR_upper vs. eMR, iSR_lower vs. eMR, and iSR_upper vs. eMR). Cells with eMR and iMR_lower both less than 0.5 – i.e., with intensity tuned suppressive and excitatory domains—lie in the lower left quadrant. Cells with eMR and iMR_lower both greater than 0.5 lie in the upper right quadrant, etc. The size of each circle is proportional to the percentage of neurons with the corresponding joint values of eMR and iMR_lower. A1 neurons with both non-intensity-tuned suppressive and excitatory domains were common (Fig. 5A,B, upper right quadrants of each plot) in contrast to the uncommonly encountered neurons with intensity tuned suppressive and excitatory domains (lower left quadrants). This suggests that the negative correlation may be chiefly due to a lack of cells with dually intensity tuned excitatory and suppressive domains. Alternatively, the apparently large percentage of neurons with non-intensity-tuned excitatory and suppressive domains might be due to the high proportion of untuned neurons in the individual distributions (Fig. 3). It might even be that the percentages of neurons with non-intensity-tuned excitatory and suppressive domains (upper right quadrant) are smaller than predicted by chance pairings of eMR and iMR values taken from the individual distributions.

Therefore we decided to determine the nature of the relationship between excitatory and suppressive domain intensity tuning more precisely with a Monte Carlo analysis. For simplicity we will only describe the specific analysis of iMR_lower vs. eMR (Fig. 5A, left), although the analogous analyses
was performed for all other inhibitory metrics. For each iMR\textsubscript{lower} value we randomly assigned an eMR value (without replacement) from one of the eMR values in the actual data. In this way the individual distributions of iMR\textsubscript{lower} and eMR were not changed, but the pairing of values was random, creating a new joint distribution. This procedure was performed 1000 times to get 1000 different joint distributions with identical individual iMR\textsubscript{lower} and eMR distributions. We then created two-dimensional 2 X 2 histograms as (such as those for the real data in Fig. 5) for all 1000 simulated data sets. The actual percentages falling in each quadrant were then compared to the distribution of 1000 simulations. The percentage of neurons in a quadrant were considered significantly below chance pairing at the p < 0.05 level if it was smaller than 95\% of the simulated values (i.e. 950/1000). The results were considered significant at the p < 0.01 level if the actual percentages were smaller than 99\% of the simulated values. This test was performed on the lower left and upper right quadrants. Based on the negative correlation of the Spearman test (Table 3), the expectation would be that either (or both) of these quadrants would have significantly fewer observations. Similar analyses were performed to determine if the percentage of neurons in the upper left and/or lower right quadrants were above chance as would be predicted by a negative correlation.

For A1 lower suppressive band iMR, the lower-left and upper right-quadrants had proportions of neurons significantly below chance (Fig. 5A, * represents significantly below chance), and the lower-right and upper-left quadrants had proportions of neurons significantly above chance (♦ in Fig. 5 represents significantly above chance). For A1 upper suppressive bands, the two lower quadrants reached significance, but the upper quadrants only approached significance (0.07 > p > 0.05). Inhibitory strength ratio (iSR), which is an index of the inhibitory strength at the highest intensity, (see methods), reached significance for all quadrants with both lower and upper suppressive bands (Fig. 5B).

**Relationship of ED-intensity tuning to suppressive domain intensity tuning in PAF**

Although for PAF neurons we also found an inverse relationship between excitatory and inhibi-
tory domain intensity tuning, the relationship was not as obvious as in A1 (Fig. 5 C,D). Spearman rank tests demonstrated that this relationship was statistically significant for lower suppressive bands, but did not reach significance for upper suppressive bands (table 3). The same method of Monte Carlo analysis was performed as in A1 to try to determine what relationships caused the significant Spearman Rank correlation, and only revealed significant deviations from chance for iSR lower in PAF. In this instance all quadrants reached significance (Fig. 5D, left).

For PAF, the data were dominated by monotonic suppressive bands, with very little intensity tuned suppression. This strong tendency likely decreased the ability of detecting trends in PAF neurons because the cells were more homogeneous in their suppressive properties. The Spearman rank analysis was also performed on combined A1 and PAF data. Overall, the introduction of PAF data increased significance (relative to A1 data alone) and had no net affect on rho values. It is hard to determine if the lack of significant correlation of PAF upper bands reflects true correlation differences between A1 and PAF, or a lack of power in PAF due to the small N (Table 3) and a small percentage (~25%) of PAF neurons with intensity tuned upper suppressive bands.

When considered together with the A1 data, these results suggest that the negative correlation between the intensity tuning of suppressive and excitatory domains is not solely due to a lack of cells with dually intensity-tuned suppressive and excitatory domains. Below chance pairing of neurons with both suppressive and excitatory domains that are not tuned for intensity, as well as above chance pairing of tuned suppressive and untuned excitatory domains and vice-versa also contribute.

*Relationship of the intensity tuning of lower and upper bands in A1 and PAF*

There was a strong correlation of the strength of inhibition between lower and upper suppressive bands. Lower band suppression that monotonically increases as a function of intensity is often accompanied by monotonically increasing upper band suppression with intensity (Fig 6). A notable aspect of the relationship between suppressive upper and lower band intensity tuning is the propensity of cells for
which both suppressive bands were untuned (Fig. 6, upper right hand quadrants). All correlations in PAF and A1 were significant with correlation coefficients in A1 ~0.36 and in PAF ~ 0.5 (Table 4).

**Relationship of excitatory domain (ED-) intensity tuning to the edges of suppressive bands**

If inputs creating suppressive sidebands are responsible for ED-intensity tuning one might expect to see a correlation between the slopes of edges of suppressive sidebands and the degree of ED-intensity tuning. Specifically one would expect that inhibitory inputs would impinge on excitatory edges at high intensities (Fig. 7A), causing the suppressive domains to slope towards the excitatory domain (Fig. 7B, Fig 1A level 2). Conversely, one would expect that for excitatory domains that are not intensity-tuned, suppressive sidebands would slope more away from them (Fig. 1B,C, level 2). To address this issue we compared the inverse slope (IS) of suppressive band edges from 5 to 45 dB above the neuron’s threshold (IS\textsubscript{5-45}), to the eMR. The IS of the lower edge of a hypothetical suppressive band with a threshold of 5 dB is shown in Fig 7C. In this case the IS\textsubscript{5-45} is –1 octaves/40 dB. Negative values denote edges slant towards lower frequencies with increasing intensity. Inverse slopes were chosen, rather than slopes because of the added stability for vertical tuning curve edges (see Sutter 2000).

In both A1 and PAF there was a correlation between the inverse slope of the suppressive band edges abutting the excitatory domain and the ED-intensity tuning (Table 5, Fig. 8). The lower suppressive band’s upper edge (LBUE, Fig. 7B) inverse slope tended to be more positive for cells with lower eMRs, i.e., more intensity tuned excitatory domains (Fig. 8). This corresponds to LBUE slanting towards the excitatory domain for ED-intensity tuned cells. Similarly, the upper suppressive band’s lower edge (UBLE) tended to be more negative for cells with lower eMRs (Fig. 8), corresponding to the UBLE slanting towards the excitatory domain for ED-intensity tuned cells. Both of these effects were significant for A1 and for PAF (Table 5). For edges that did not abut the excitatory domain (the lower edge of the lower band (LBLE) and the upper edge of the upper band (UBUE)) the correlation never reached significance (Table 5). These results are consistent with the notion that ‘surround’ inhibition
impinging on the excitatory tuning curve helps to create ED-intensity tuning.

**The relationship of frequency separation of suppressive and excitatory domains to ED-intensity tuning**

The model of Fig. 1 predicts the above result that intensity-tuned excitatory domains would have suppressive sidebands with steeper slopes towards the BEF (Fig. 1A vs. 1C). This reflects the notion that inhibitory inputs overlap with the excitatory domain at high intensities. The model’s predictions for low intensities are less clear. On the one hand, the assumption that all inhibition is similar strength (or alternatively if the slopes of the edges of inhibitory inputs were all the same) would lead to the model predicting that for cells with strong ED-intensity tuning, the best frequency of adjacent suppressive bands (BIF) would be closer to the BEF. The logic is that when the BIFs are far from the BEFs, the inhibitory inputs would be less likely to overlap with BEF excitatory inputs at high intensities; therefore this would predict monotonically increasing responses as a function of BEF tone intensity (Fig. 9A). When the BIFs are closer to the BEF (Fig. 9B), inhibitory inputs of equivalent bandwidth would overlap with the BEF excitatory inputs at higher intensities, thereby causing the firing rate to decline for higher intensity BEF tones (inhibitory inputs aligned with double arrow at BEF in Fig. 9B).

Alternatively, it could be that frequency separation between inhibitory and excitatory input at low intensities is not a major factor in the model when compared to slopes. To distinguish these two possibilities we examined the relationship between eMR and the frequency separation of the suppressive and excitatory domains at both low (threshold) and high (45 dB above suppression threshold) intensities.

First, we investigated the correlation between ED intensity tuning and the differences in the BFs of excitatory and suppressive domains. For lower and upper A1 and PAF suppressive bands, no significant correlations were found and no trends were observed (Table 5). It is also interesting to note that the separation between the BIF of the upper band and the BEF in PAF was larger than in A1 (PAF median = 0.65 octaves, A1 median = 0.34 octaves, Mann Whitney: U p = 0.0064). This trend also argues against further BEF-BIF separation corresponding to less ED-intensity tuning, as PAF has more ED-
tuning but has upper suppressive bands whose BIFs are further from the BEFs.

Neurons whose suppressive domains hug the edge of the excitatory domain (for example, Fig. 10) can provide insight into the lack of relationship between BEF-BIF separation and ED-intensity tuning. In this cell with an eMR of 0.73, the BEF and BIFs are similar, but both suppressive bands slant away from the excitatory domain, suggesting that there is competition between excitation and inhibition and the dominance of one over the other is independent of the BEF-BIF difference. The inverse relationship between suppressive and ED-intensity tuning also supports this idea.

This result does not necessarily contradict the model (but infers that assumptions of equal strength of all inhibitory bands is not warranted). The critical aspect in the model is not the frequency of the inhibition at low intensities, but the frequency and strength of inhibitory inputs at high intensities, where responses to BEF tones start to decline for intensity-tuned excitatory domains. This is because at suppression threshold two-tone suppression/inhibition reflects only the most sensitive unit, not necessarily the strongest.

To test whether the high intensity suppressive domains correlate with ED-intensity tuning (and the observed results with inverse slopes), we investigated the relationship between ∆UBLE and eMR. ∆UBLE (Fig. 9C,D) is calculated by taking the frequency difference (in octaves) between UBLE 45 dB above threshold and the BEF. For both A1 and PAF a significant correlation (Table 5) was observed between ∆UBLE and eMR, supporting the notion that high intensity inhibition is responsible for ED-intensity tuning. A significant correlation (Table 5) was also observed between ∆LBUE and eMR. The results of this section are consistent with the model. When the edges of strongly sloping inhibitory inputs (Fig. 1A level 1 and red lines) intrude into the BEF of the input, an intensity tuned excitatory domain is created. An outcome of this interaction is that the edge of the suppressive domain (Fig. 1A level 2) gets closer to the BEF.

**DISCUSSION**
Mechanisms underlying intensity tuning: fast inhibition and delayed excitation

These results are consistent with the working hypothesis that inhibitory input neighboring the excitatory edges of frequency tuning curves is responsible for excitatory domain intensity tuning by inhibiting BEF responses at higher intensities. Supporting this is (1) the inverse relationship between excitatory and neighboring suppressive domain intensity tuning, and (2) the relationship between the slopes of suppressive band edges and ED-intensity tuning.

In addition to these results, other work strongly constrains the properties of inhibitory input carving out ED-intensity tuning. In particular the inhibition must be (1) effective at high intensities and (2) strong and/or fast. Intensity dependent inhibition consistent with (1) have been reported throughout the auditory system, with midbrain and brainstem binaural processing providing especially compelling examples (Yin et al. 1985; Sanes 1990; Park et al. 1996; Park et al. 1997). Fast inhibition (2) is required because the highly phasic onset response of many cortical cells contrasts with the more sustained responses of many sub-cortical neurons. Accordingly, the inhibitory post-synaptic potentials (ipsps) responsible for removing onset responses at high intensities must affect the cell before excitatory post-synaptic potentials can bring the neuron past action potential threshold.

The inhibition responsible for the observed cortical intensity tuning need not be localized to cortex. Both fast ipsps and slow epsps consistent with these results have been demonstrated throughout the auditory system (e.g., brain stem Wu & Kelly 1996; Ferragamo et al. 1998; midbrain: Nelson & Erulkar 1963; Carney & Yin 1989; Covey et al. 1996; Kuwada et al. 1997; cortex: Bartlett & Smith 1999). This inhibition of onset responses by effective ‘feed-forward’ inhibition is a relatively unique property of the auditory system when compared to other senses.

So how can the auditory system create this effective ‘feed-forward’ inhibition? This can be produced either anatomically (Fig. 1) or physiologically. Anatomically fast shorter latency feed-forward inhibitory pathways, and/or delayed excitatory pathways could create this fast inhibition. There is evi-
ence for such feed-forward inhibition or delayed excitation in projections from brain stem to midbrain (Adams & Mugnaini 1984; Shneiderman et al. 1988; Bauer et al. 2000; Vater et al. 1997; Moore et al. 1998; Zhang et al. 1998) and from IC to MGB (Winer et al. 1996; Saint Marie et al. 1997; Peruzzi et al. 1997; Bartlett & Smith 1999), as well as within the brainstem and cortico-cortical projections. Feed-forward inhibition can also be achieved physiologically. For example a combination of weak excitatory and strong inhibitory input could allow the inhibition to delay or prevent the neuron from crossing action potential threshold. Similarly if ipsps are integrated (in time and space) more efficiently than epsps, inhibitory inputs can exert a stronger influence before action potential threshold is crossed. Faster receptor dynamics and/or transmitter release for inhibition could also generate the same effect.

There is physiological evidence for such mechanisms, although it is difficult to segregate these physiological effects from anatomical contributions (e.g., brainstem Zhang & Oertel 1994; Grothe & Sanes 1993; cortex Hefti & Smith 2000; Cox et al. 1992).

**Mechanisms underlying intensity tuning: multiple stages of inhibition**

The constraint that inhibition must be fast to create ED-intensity tuning can help resolve the seemingly contradictory results of this study and those of Calford and Semple who found intensity tuning of excitatory and suppressive domains were positively correlated (Calford & Semple 1995). In other words they found that neurons with intensity-tuned excitatory domains also had intensity tuned inhibitory domains, and cells with non intensity tuned excitatory domains also had un-tuned inhibitory domains. In contrast to our study which used simultaneous masking to characterize suppressive domains, Calford and Semple used forward masking where the putative inhibitory tone was always presented prior to a BEF tone with no temporal overlap, i.e., no time when both tones were on. In our study the suppressive and BEF tones were gated together and always simultaneously presented, completely overlapping. So why do results of these two studies seem opposite? One possible explanation is that the inhibitory input responsible for creating intensity tuning is separable and distinct from that responsible for
forward masked inhibition (Calford & Semple 1995).

In particular, one can think of two-stages of inhibition: a fast ‘feed-forward’ inhibition which creates intensity tuning; and a longer latency inter-neuron mediated inhibition responsible for forward masking. By making reasonable assumptions about the interneuron inhibition, such as cells with similar excitatory domain intensity tuning laterally inhibit each other, one can account for the results of both studies. Figure 11 presents a model that incorporates both fast and slow inhibition and accounts for both the presented and the forward masking data. Level 1 and Level 2 were presented in the introduction (Fig. 1). The fundamental premise of this model is that ED-intensity tuning first is created by inhibition, and then ED-intensity tuned cells laterally inhibit each other. The model has three successive levels. Level one neurons are connected to level two neurons via two pathways: a mono-synaptic fast-feed forward lateral inhibitory pathway; and a delayed excitatory pathway (the delay is schematized by going through two synapses). Level 3 neurons laterally inhibit each other. The levels refer to successive stages of processing which could be connections within or between brain areas.

Fig. 11A shows that the convergence of an array of broadly tuned inputs creates a level 3 cell with intensity tuned excitatory and forward masked suppressive domains (Fig. 11D quadrant III), but with simultaneous two-tone suppressive domains that are not intensity-tuned (Fig. 11D quadrant iv). Intensity tuned excitatory domains at level 2 are caused by the broad frequency tuning of the level 1 inputs, some of which create broad lateral inhibition at level 2. This inhibition is particularly strong at high intensities where the low frequency tail of the upper band’s inhibitory input infringes on the cell’s BEF (Fig. 11A level 2). Therefore, level two neurons have intensity-tuned excitatory domains and broad sloping simultaneous two-tone suppressive domains that are not intensity tuned.

An essential feature of this model is that neurons with similar ED-intensity tuning cluster together and laterally inhibit each other, whereas cells with dissimilar ED-intensity tuning do not. This lateral inhibition, delayed by going through an interneuron (Fig. 11A, blue dashed line), can be found in
level 3. In Fig. 11A this creates intensity tuned forward-masked suppressive domains. For simplicity we have shown this interneuron in level 3, but it also could be in the same brain area that hosts the cells of level 2 or could be mediated through feedback from level 3 to level 2. Evidence supports all of these possibilities (e.g., He 1997; Shofner & Young 1987; Jen & Zhang 1999; Suga et al. 2000; Moore et al. 1998; Paolini et al. 1998; Rhode 1999; Wickesberg & Oertel 1990; Winer et al. 1995; Zhang & Oertel 1994; Zhang et al. 1997), which certainly are not mutually exclusive. Rather than focusing on where the inhibition occurs, we wish to focus on the essential feature of this model that cells with similar ED-intensity tuning cluster together and laterally inhibit each other, whereas cells with dissimilar ED-intensity tuning do not laterally inhibit each other. Neurons with similar ED-intensity tuning spatially cluster in auditory cortex (Imig et al. 1990; Schreiner et al. 1992; Heil et al. 1994; Clarey et al. 1994; Sutter & Schreiner 1995), possibly providing a site for such lateral inhibition. Although, to our knowledge clustering of neurons with similar ED-intensity tuning have not been reported sub-cortically, there is evidence that certain brain areas have a higher proportion of ED-intensity tuned cells (e.g., DCN: Voigt & Young 1980; caudal MGB: Rodrigues-Dagaeff et al. 1989). Finally feedback connections are topographic with respect to several information bearing parameters of sound (Yan & Suga 1999; Zhang et al. 1997; Suga et al. 2000). If topography also holds with respect to ED-intensity tuning, this could be a source of delays in forward masked lateral inhibition.

The model assumes segregated parallel pathways for neurons with intensity-tuned (Fig. 11A) and un-tuned (e.g., Fig. 11B) excitatory domains. In the pathway with non-intensity tuned excitatory domains of Fig. 11B, broad inputs and weak inhibition at level 2 allow for the ED to remain un-tuned, because the inhibition does not have the strength to significantly influence the BEF response. Inhibitory domains are intensity tuned because the excitation is strong enough to overcome inhibition at the best inhibitory frequency at higher intensities. Since the excitation is untuned responses using simultaneous masking in this pathway should fall in quadrant ii. Strong inhibition at level 3
of neurons in this segregated un-tuned pathway creates un-tuned forward masked inhibition (Blue), and because the ED is also untuned responses in forward masked quadrant I.

The results with respect to the model of Fig. 11C is a bit more complex. In this model, inputs with narrow frequency tuning allow for non intensity tuned excitatory domains in level 2 because the lateral inhibitory inputs do not extend to the BEF. While there is a high proportion of cells with non-intensity tuned excitatory and suppressive domains (quadrant i), the proportion of cells is lower than one would predict by chance pairings of the individual distributions of eMR and iMR. This indicates that the high proportion of cells in quadrant i is solely due to the large percentage of neurons with non-intensity tuned domains. This supports that level 3 neurons with these properties exist in high proportion, but that at the cortical level, interaction between inhibitory and excitatory domains (potentially via the model of Fig. 11A and B), is serving to overcome this, possibly due to mechanisms ascribed to Fig. 11A and B. The data argues that even in the non-intensity tuned EDs, interactions between overlapping frequencies in the inhibitory and excitatory input is prevalent.

This relatively simple model explains the results of Calford and Semple using forward masking and of the present study using simultaneous two-tone masking. A critical assumption built into this model is that there are segregated parallel pathways for neurons with intensity-tuned and un-tuned excitatory domains. By restricting level 3 flanking inhibition to cells within a pathway the results of Calford and Semple can be explained. Fig. 11A level three neurons have intensity tuned excitatory and forward masked suppressive domains, and Fig. 11B level three neurons have non-intensity tuned excitatory and forward masked suppressive domains. With regard to our simultaneous two-tone suppressive results, the model consistently predicts the types of correlation described in Fig. 5. In particular the model predicts a lack of neurons with both intensity tuned excitatory and simultaneous masked suppressive domains (Fig. 11D, quadrant iii). When the inputs are broad (Fig.
11A), excitatory domains are intensity-tuned and simultaneous two-tone suppressive domains are not (Fig. 11D quadrant iv)-- a condition that was quite common, particularly in PAF. The other common condition (by overall proportion and by above-chance pairing of individual eMR and iMR distributions) of intensity-tuned simultaneous two-tone suppressive and un-tuned excitatory domains (Fig. 5 upper left quadrants of bubble histograms) can be explained by broad inputs and weak inhibition at level 2, followed by strong inhibition at level 3 (Fig. 11B). Strong inhibition is required at level 3 to keep the results consistent with Calford and Semple’s that neurons without intensity tuned excitatory domains also have un-tuned forward masking inhibitory domains. In neural network simulations, it has been our experience that the model structure of Fig. 11 does not produce both intensity tuned simultaneous two-tone suppressive and excitatory domains in the same level 3 neurons (personal observation); a finding which is consistent with the results (Fig. 5, lower left quadrants). Finally, because only cells with similar excitatory domain intensity tuning laterally inhibit each other, this model also predicts a lack of cells with oppositely intensity tuned excitatory and forward-masked-inhibitory domains which is consistent with Calford and Semple.

In conclusion, we believe the most parsimonious explanation of our results and those of Calford and Semple is separate stages of inhibition carving out ED-intensity tuning and creating forward masked suppressive domains. Calford and Semple made similar arguments based on the strong evidence from their studies that forward masking inhibition and the inhibition responsible for intensity tuning were the result of two independent inhibitory mechanisms.

Mechanisms underlying intensity tuning: Loci of inhibition

With the current evidence it is impossible to assign a specific brain area corresponding to levels 2 and/or 3 of the model. Pharmacological experiments indicate that excitatory domain intensity tuning is created de novo in many auditory areas (e.g., CN: Caspary et al. 1979; Evans & Zhao 1993; Davis & Young 2000; IC: Faingold et al. 1991; Fuzessery & Hall 1996; Vater et al. 1992; Pollak & Park 1993;
Yang et al. 1992; Lu & Jen 2001; Wang et al. 2000; MGB: Suga et al. 1997; A1Chen & Jen 2000). Although a role for cortical inhibition in shaping intensity tuning has not been reported for non-echolocating mammals, the first published combined pharmacological and physiological studies in chinchilla auditory cortex report an inhibitory role in shaping excitatory domains (Wang et al. 2000; 2002). However because ED-intensity tuning at the brainstem, and midbrain level can result from inhibition of sustained responses without affecting the onset responses, it is unclear if a narrower subset of auditory pathway neurons is responsible for the ED-intensity tuning observed in cortex, which require fast inhibition to remove onset responses. More refined studies of the timing of inhibition creating ED-intensity-tuning in different brain areas is required.

There are also several areas which might be the site(s) of delayed, possibly lateral, inhibition that carves out cortical forward-masked areas (Calford & Semple 1995; Brosch & Schreiner 1997); inhibitory interneurons exist in many brain structures (Winer & Larue 1989; 1996). However the topography of cortical intensity-tuned neurons makes cortex a prime candidate.

Limitations on interpretation due to the use of simultaneous two-tone masking

Although suppressive spectral receptive field properties have been reported using simultaneous two-tone masking (e.g., Suga 1965; 1968; Suga & Tsuzuki 1985; Zhang & Feng 1998; Zhang et al. 1999; Fuzessery & Feng 1982; Fuzessery & Hall 1996; Fuzessery 1994; Ehret & Merzenich 1988b; Sutter & Schreiner 1991; Sutter et al. 1999), to our knowledge the only previous study of the relationship between the frequency extent of suppressive sidebands and ED-intensity tuning that directly measured suppressive bands used forward masking (Calford & Semple 1995). This choice was at least partially because the use of simultaneous two-tone stimulation introduces inherent limitations in the interpretation of neural inhibitory contributions due to the well-established mechanical effect of simultaneous two-tone suppression (TTS), (Sachs & Kiang 1968) in the basilar membrane (Ruggero et al. 1992; Rhode 1977).
While the simultaneous paradigm poses some problems, its use is essential to investigate the role of inhibition in shaping ED-intensity tuning, because cortical ED-intensity tuning requires fast inhibition. The most common models of mechanisms underlying ED-intensity tuning support this. For example, a common argument made is that ED-intensity tuning might result from “spectral splash or splatter”, caused by the rapid onset of BEF tone pips, extending the tone-pips’ power spectrum into lateral inhibitory sidebands (Phillips 1988; Phillips et al. 1995). Another possible mechanism is that tails of inhibitory sideband input might impinge on excitatory areas. Finally inhibitory input with BIF matched to the BEF, could create ED-intensity tuning if the inhibition becomes more effective at higher intensities. For all of the above-mentioned mechanisms that could create ED-intensity tuning an essential condition is that there be inhibition fast enough to eliminate onset responses. Therefore, when a single loud BEF tone is presented, in addition to excitatory inputs being activated, inhibitory inputs also must be simultaneously activated. Because a single tone simultaneously evokes both the excitatory and inhibitory inputs, the spectral properties of inhibition under these conditions can only be completely explored by using simultaneously shaped and presented BEF and inhibitory tones.

Because we used simultaneous two-tone stimuli it is possible that the observed suppressive domains simply reflects basilar membrane TTS. While it is likely that TTS contributes to some of the observed results, several lines of evidence argue that neural inhibition is involved. The bandwidths, shapes, thresholds, and strength of cortical two-tone suppressive domains often are quite distinct from peripheral TTS (Sutter et al. 1999; Loftus & Sutter 2001b). The time course of cortically observed inhibition (studied using simultaneous, overlapping, and forward masking and varying the delay of the onset of probe tone relative to the masker) is also incompatible with pure TTS (Brosch & Schreiner 1997; Loftus & Sutter 2001a). The properties of suppression reported herein further argue against pure TTS. For example, the frequency separation between excitatory and suppressive domains is further than expected from peripheral TTS. Additionally, the relationship between suppressive band edges and ED-
intensity tuning is not expected if TTS were solely being measured. The a priori assumption would have to be that TTS is not related to excitatory domain intensity tuning, since TTS is not observed with a single tone and intensity tuning is not seen in the basilar membrane. Even in cases where suppressive domains share some TTS properties (e.g., in Fig. 10 the threshold and frequency extent of the lower and upper suppressive bands nearest the excitatory area are consistent with two-tone suppression), other properties such as the intensity tuning of the upper suppressive band are inconsistent with peripheral TTS. In fact, some cortical cells (even some that are quite narrowly tuned) do not show any two-tone suppressive domains, whereas TTS is ubiquitous in auditory nerve fibers. In aggregate these data indicate that (1) peripheral TTS is not solely responsible for the observed effects and (2) under some conditions the effects of TTS with a BEF probe tone cannot even be seen at the cortical level, presumably due to excitatory integration and properties of synaptic transmission (such as threshold).

Excitatory domain(ED-) intensity tuning reflects inhibition at high intensities

It was a bit surprising that suppressive domains with BIFs close to the BEF did not appear to be closely associated with ED-intensity tuning, particularly because the slopes of suppressive sidebands’ edges were correlated with ED-intensity tuning. This result is even more surprising since in the inferior colliculus when the BEF and forward masked suppressive domain BIFs of a cell were close, lateral inhibition was more effective in shaping location sensitivity (Zhou & Jen 2000).

There are good explanations, though, for the results we observed. First, a neuron’s spectral receptive field is not a clear picture of distinct separable inhibitory and excitatory inputs, but rather represent the net sum of excitatory and inhibitory inputs at each frequency. In this context one can think of the spectral receptive field, particularly near borders of excitatory and suppressive domains, as resulting from competition between excitatory and inhibitory inputs. For ED-intensity tuning, which depends on the competing influences of excitatory and inhibitory inputs at high intensities, it is not necessarily the most sensitive inhibitory inputs that are important, but the number and strength of inhibitory inputs at
higher intensities. Second TTS, which does not play a role in the creation of intensity tuning, is expected to have BIFs very close to BEFs (e.g., See Sachs and Kiang 1968). Therefore, in cells with less of an influence of neural inhibition (and therefore less intensity-tuned excitation), one might expect the peripheral TTS properties to dominate the suppressive domain near threshold. Therefore, when using simultaneous two tone masking and recording from cells with little neural inhibition, one might expect to find best ‘inhibitory’ frequencies very close to the excitatory BF, due to the presence of TTS.

In conclusion, when looking at excitatory domain intensity tuning one must account for inhibition at high intensities. Using low intensity threshold measures (such as best frequency) and assumptions about inhibitory domain shape are not necessarily reliable.

Habituation

Aside from the interpretational limitations introduced by TTS, using two-tone masking poses another limitation. The need to drive a cell repetitively (>= 675 times) with a BEF tone biases the recorded samples to cells that can be reliably repetitively driven. This means cells with weak responses or that rapidly habituate to BEF tones will be under sampled. However, these are exactly the cells that are most likely to have strong inhibition (since inhibition can cause weak responsiveness or habituation). Table 2 suggests that in PAF this type of bias was present because ED-intensity-tuned cells (cells presumably experiencing stronger inhibition, and therefore potentially not having intensity-tuned suppressive domains) were under-sampled with the two-tone paradigm. This effect was not observed in A1. This could partially explain why, when not using paired comparisons, the strength and lack of suppressive domain intensity tuning in PAF neurons was not as large as one might expect given the differences in ED-intensity tuning between A1 and PAF (Table 1). However paired comparisons (Table 3) revealed the relationship between excitatory and suppressive domain intensity tuning in PAF.

Binaural Vs. Monaural

In PAF we used binaural stimulation. This could effect the proportion of recorded intensity-
tuned neurons. For example (Klug et al. 1999) show fast ipsilateral inhibition in the IC putatively from a feed-forward pathway from the lateral lemniscus; such feed-forward inhibition could contribute to ED-intensity tuning. Also, ED-intensity tuning can vary with different binaural stimulating conditions (Semple & Kitzes 1993a). Potential binaural stimulating differences are mitigated by two effects: (1) while overall response magnitudes change with binaural stimulating conditions, ED-intensity tuning in PAF does not seem to change (Orman & Phillips 1984); (2) the 90 degree contralateral stimuli used in PAF would create substantially weaker ipsilateral stimulation than a stimulus presented at equal intensities to the two ears (the most common binaural stimulating condition.)

**Potential Anesthetic Effects**

Pentobarbital may have an effect on these studies by potentiating the effect of GABAergic inhibition. The usual manifestation is reduced spontaneous activity, and changes in temporal response patterns. Studies of excitatory and inhibitory response properties in cat and monkey primary auditory cortex indicate that barbiturate anesthesia does not have a significant effect on frequency, intensity and temporal tuning (Pfingst et al. 1977; Pfingst & O’Conner 1981; Merzenich et al. 1984; Stryker et al. 1987; Calford & Semple 1995), even though the anesthetic non-specifically decreases cortical activity. For interpretation, however, we cannot rule out that the anesthetic used has some effect on the results. Therefore, all interpretations should be made cautiously.

**Implications of upper and lower band correlation**

That upper and lower band iMRs tended to co-vary is interesting because it indicates that the creation of these two bands are not independent. There are several possible ways this could happen. One way is that upper and lower bands, ostensibly result from one broad inhibitory input which extends beyond both lower and upper edges of the excitatory frequency tuning curve. Another possibility is that the upper and lower bands are derived from two distinct inhibitory inputs; but that the inputs are matched in their intensity tuning, possibly because of the known clustering of similarly intensity tuned
cells in the auditory system.

In conclusion, by measuring fast inhibition in A1 and PAF we were able to demonstrate that intensity tuned excitatory domains are inversely related to the cells’ suppressive domain intensity tuning. The results are consistent with a model of multiple independent inhibitory mechanisms and parallel segregated intensity-tuned and un-tuned pathways in the central auditory system.
FOOTNOTES

1 Accordingly, a careful distinction between excitatory inputs, and the tuning of the net responses to these inputs is required. When we refer to a portion of the frequency response area (FRA) that has a net excitatory effect, we will use the term “excitatory domain”. This refers to the measured response and helps to distinguish the tuning of the entire area from the excitatory input, which might have different tuning properties. The same nomenclature holds for suppressive domains and inputs, except suppressive domains may also be referred to as suppressive ‘bands’ (Fig. 1). The word ‘suppressive’ is used rather than inhibitory so as not to confuse the domains to the mechanisms that might create them; it is not intended to imply the mechanisms of two-tone suppression in the basilar membrane.

Furthermore henceforth when we refer to what historically are called ‘nonmonotonic’ or ‘intensity tuned’ neurons, we will use the nomenclature excitatory domain (ED) intensity tuned neurons to distinguish this type of intensity tuning from intensity tuning of the inhibitory domain, which will be called inhibitory domain intensity tuning.

2 The term inhibitory is used here to keep the nomenclature consistent with previous literature (e.g., Sutter et al, 1999)

3 Note: lateral inhibition is not required to create intensity tuning. There are several other possibilities including high-threshold inhibition matched in BIF to the BEF. However the inhibition must be fast relative to the excitation.
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FIGURE LEGENDS

Figure 1: (A) shows possible mechanism creating cells with intensity tuned excitatory domains and non intensity-tuned suppressive domains. Level 1 shows broadly frequency tuned input neurons (circles). To the left of the circles are the frequency response areas (FRAs) of the corresponding neurons. At level 2, broad fast lateral inhibition (red feed forward connections) inhibits responses at the best excitatory frequency (BEF) at high intensities. This creates cells with intensity tuned excitatory domains, but un-tuned suppressive domains (gray shading in the FRAs to the right of level 2 neurons). (B) Shows a possible mechanism for creating neurons with inhibitory domains that are intensity tuned at the best inhibitory frequency (BIF), but excitatory domains that are not intensity tuned. All connections are the same as in A. All that changes is the strength of inhibition. (C) Shows a possible a mechanism for creating cells with excitatory and inhibitory domains that are not tuned for intensity. All connections are the same as in A. All that has changed is the frequency tuning of the inputs. Note: we have used lateral inhibition in the model for simplicity. All of the observed properties could be created with surround inhibition matched in frequency to the excitation.

Figure 2. Relationship of monotonicity and strength ratios to six response vs. intensity functions. A lower monotonicity or strength ratio means a function is more sharply tuned. On the left (A-C) the relationship of the excitatory monotonicity ratio (eMR) to single tone response vs. intensity functions are shown. Single tone responses at the best excitatory frequency (BEF) were used. The relationship is shown for a neuron with a monotonically increasing function (A), an intensity tuned function (C), and a function with weak intensity tuning (B). On the right (D-F) the relationship of the inhibitory strength ratio (iSR) and inhibitory monotonicity ratio (iMR) to two-tone response vs. intensity functions are shown for three neurons. In this case the response vs. intensity function is taken from simultaneous two tone stimulation with one tone at each cell’s BEF and one at the cell’s best inhibitory frequency (BIF). The intensity of the BIF component is varied and the BEF tone intensity is fixed at 5-20 dB
above threshold. The dashed lines represent the response to the BEF tone presented by itself. In (D) a cell with monotonically increasing inhibitory strength as a function of intensity is shown. Note that monotonically increasing inhibitory strength corresponds to a monotonically decreasing function because a larger decrease in response corresponds to stronger inhibition. In (F) a cell with an intensity tuned suppressive domain is shown and in (E) an intermediate case is shown.

**Figure 3.** Histograms of eMR and iMR distributions for different bands in A1 and PAF.

**Figure 4.** The relationship of suppressive to excitatory domain intensity tuning in A1. Scatter plots are shown for lower and upper suppressive bands vs. eMR. Every point corresponds to one neuron. Statistical significance of Spearman Rank Correlation test is shown in the upper right hand corner of each plot.

**Figure 5.** Bubble histogram demonstrating the non-linear correlation of suppressive to excitatory domain intensity tuning in A1. The size of the bubbles (circles) correspond to the percentage of cells with combinations of monotonicity and strength ratios given by the intersection of the x and y axis.

(A) A1 iMR vs. eMR for upper and lower suppressive bands. The histograms are binned into 4 quadrants by ratios of greater or less than 0.5. The lower left quadrant represents neurons with low eMR and iMR values, that is, cells that have intensity tuned excitatory and suppressive domains. The percentages of cells in each quadrant were statistically compared to random pairings from the individual eMR and iMR distributions with a Monte Carlo Analysis. If the proportion of cells fall significantly below the expectation with random pairings at the p <0.05 level an * is placed in the corner. If the significance is at the p<0.01 level ** was used. If the proportion of cells is significantly above the expectation with random pairing at the p <0.05 level an ◆ is placed in the corner, ◆◆ for p < 0.01. (B) Same plot for iSR in A1. (C-D) Similar plots for PAF.
Figure 6. Three dimensional bubble histogram demonstrating the non-linear correlation of upper and lower suppressive band intensity tuning. The left column plot represents data from A1 and in the right column from PAF.

Figure 7. Schematized tuning curve showing a possible mechanism of how inhibitory inputs might combine to create intensity tuning, and the terminology of edges of bands, and how IS\textsubscript{5-45} is measured. (A) shows putative inhibitory inputs with broad sloping edges which infringe on the BEF. Dark solid line outlines the excitatory tuning curve of the input. Shaded gray areas mark the putative tuning curves of the inhibitory inputs. Darkest shaded area corresponds to frequencies at which the lower and upper inhibitory inputs overlap. (B) shows the predicted excitatory and suppressive domains of an output neuron receiving the input from A. Dark solid line outlines the excitatory domain. The BEF is represented by a vertical dashed line. Dark areas demarcate the inhibitory bands. Arrows point to various edges of the tuning curve indicating what they are called. Note that the Lower suppressive band’s upper edge (LBUE) and upper band’s lower edge (UBLE) neighbor and impinge on the excitatory frequency tuning curve. The lower band’s lower edge (LBLE) and upper bands upper edge (UBUE) do not neighbor the excitatory frequency tuning curve. Note that this schematic is for a neuron with a circumscribed excitatory domain. (C) shows how IS\textsubscript{5-45} was calculated from the lower edge of a suppressive band. Note that this band’s threshold was 5 dB and IS\textsubscript{5-45} would be -1 octave/40dB.

Figure 8. Plots demonstrating the relationship of the inverse slope of suppressive band edges abutting the excitatory domain and excitatory domain (ED-)intensity tuning. Each plot shows the relationship between a suppressive band edge’s IS\textsubscript{5-45} (inverse slope between 5 and 45 dB above threshold) and the eMR.

In the top box labeled A1 the different plots show the relationships of the for 4 different suppressive band edges to eMR in A1. The lower suppressive band’s upper edge (LBUE) and upper band’s lower edge (UBLE) abut the lower and upper edge of the excitatory tuning curve respectively, whereas the
lower suppressive band’s lower edge (LBL) and the upper band’s upper edge (UBL) are distant from the excitatory tuning curve. Note that the two abutting edges ISs vary greatly with eMR, but the non-abutting edges do not (Table 6). The middle box shows the same relationships for PAF, and the lower box shows the relationships for combined A1 and PAF data.

**Figure 9.** Schematic showing a possible relationship between the BEF-BIF separation and ED-intensity tuning. Solid outline depicts the excitatory domain and shaded areas represent suppressive domains; dashed lines represent BEFs and BIFs. (A) shows the frequency tuning of two inhibitory inputs with distant BIFs from the excitation (arrows). In this case, because the BIFs are far in frequency from the BEF the inhibitory inputs do not impinge on the excitatory input. Therefore the excitatory domain is not intensity tuned. (B) shows the expectation when the BIF of the inhibitory input (and therefore the measured suppressive domain) is close to the BEF. In this case, the inhibitory input impinges on the excitatory input at high intensities and both inhibitory inputs overlap at the BEF creating strong inhibition and ED-intensity tuning. (C-D) shows how high intensity inhibition could correlate with intensity tuning, independent of BEF-BIF separation. In these plots, excitatory and suppressive response domains are shown rather than the inputs, as in A and B. In (C), the suppressive bands are distant at higher intensities, whereas in (D) it abuts the excitatory domain. The frequency difference, ΔLBL, between the LBL and the BEF is shown in (C). The analogous difference, ΔUBL, is also shown in (C).

**Figure 10:** Suppressive and excitatory domains of an A1 neuron with BIFs close to the BEF. This cell did not have strong ED-intensity-tuning.

**Figure 11.** Our results when combined with those of Calford and Semple support two distinct stages of inhibition. One creates ED-intensity tuning (level 1 to level 2 in A). In the other ED-intensity-tuned cells laterally inhibit each other (level 3 in A). This stage is like a segregated ED-intensity-tuned 'channel' where ED-intensity-tuned cells only inhibit each other, but do not interact with non intensity-tuned
neurons. All effects of the first stage are shown in red; all of the second stage in blue. This model architecture can explain all the results observed by this and Calford and Semple's study. Note the phrase two-tone inhibitory (TTI) domain is used in this figure to be consistent with the literature, because the term two-tone suppression is historically used to refer to the peripheral suppression in the basilar membrane.

(A) shows a possible mechanism creating cells with intensity tuned excitatory domains, un-tuned simultaneous masked suppression, and intensity tuned forward masked suppression. Level 1 shows broad excitatory frequency tuning curves (left) of different input cells (circles). At level 2, broad, fast lateral inhibition (red feed forward connections) inhibits responses at the best excitatory frequency at high intensities. This creates cells with intensity tuned excitatory domains, but non intensity tuned simultaneous masked suppression (tuning curves to the right of level 2 cells). At level 3, cells with intensity tuned excitatory domains laterally inhibit each other (blue dashed lines) creating intensity tuned forward masked suppression. The reason this intensity tuned inhibition would not be seen with simultaneous masking is because this inhibition experiences typical synaptic delays.

(B) shows a possible mechanism for creating cells that are not intensity tuned for excitatory or forward masked suppressive domains, but are intensity-tuned for simultaneous masked suppression. All connections are the same as in A. All that changes is the strength of inhibition.

(C) shows how cells that have both excitatory and suppressive domains that are not intensity tuned could be formed. All connections are the same as in A. All that has changes is the frequency tuning of the inputs.

(D) Schematic figure similar to the plots in Fig. 5 summarizing the results of this paper using simultaneous masking and Calford and Semple’s paper using forward masking. Each quadrant is marked with roman numerals (lowercase for simultaneous two-tone suppression, and uppercase for forward masked inhibition). Quadrant numerals are also placed next to the frequency tuning curves shown in A-C.
### Table 1: Comparison of intensity tuning between PAF and A1

<table>
<thead>
<tr>
<th></th>
<th>Excitatory domain</th>
<th>Lower suppressive band</th>
<th>Upper suppressive band</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>eMR</td>
<td>iMR</td>
<td>iSR</td>
</tr>
<tr>
<td>A1 PAF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of strongly tuned neurons</td>
<td>33.0%</td>
<td>58.7%</td>
<td>41.7</td>
</tr>
<tr>
<td>% of intermediately tuned neurons</td>
<td>29.1%</td>
<td>23.9%</td>
<td>15.8</td>
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<tr>
<td>% of monotonic neurons</td>
<td>37.8%</td>
<td>17.4%</td>
<td>42.5</td>
</tr>
<tr>
<td>ratio 50th% (median)</td>
<td>0.69</td>
<td>0.44</td>
<td>0.69</td>
</tr>
<tr>
<td>X²</td>
<td>p &lt; 0.0001 **</td>
<td>p = 0.580</td>
<td>p = 0.516</td>
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<tr>
<td>Mann-Whitney</td>
<td>p &lt; 0.0001 **</td>
<td>p = 0.439</td>
<td>p = 0.472</td>
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<tr>
<td>N</td>
<td>230</td>
<td>92</td>
<td>117</td>
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</tbody>
</table>

- **Strongly tuned** is defined as a monotonicity or strength ratio <= 0.5
- **Intermediately tuned** is defined as 0.5 < ratio < 0.8
- **Monotonic** is defined as a monotonicity or strength ratio >= 0.8
- **comparing A1 and PAF**

* p < 0.05, ** p < 0.01
Table 2: Relationship of intensity tuning to the ability to collect a two-tone FRA

<table>
<thead>
<tr>
<th></th>
<th>PAF excitation</th>
<th>A1 excitation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>2-tone</td>
<td>no 2-tone</td>
</tr>
<tr>
<td>% of strongly tuned neurons</td>
<td>49.1%</td>
<td>74.3%</td>
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<tr>
<td>% of intermediately tuned neurons</td>
<td>28.1%</td>
<td>17.1%</td>
</tr>
<tr>
<td>% of monotonic neurons</td>
<td>22.8%</td>
<td>8.6%</td>
</tr>
<tr>
<td>monotonicity ratio 50th% (median)</td>
<td>0.51</td>
<td>0.36</td>
</tr>
<tr>
<td>$X^2$</td>
<td>p = .051</td>
<td>p = .363</td>
</tr>
<tr>
<td>Mann-Whitney</td>
<td>p &lt; .05</td>
<td>p = .702</td>
</tr>
<tr>
<td>N</td>
<td>57</td>
<td>35</td>
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Table 3: Relationship of eMR to inhibitory measures

<table>
<thead>
<tr>
<th></th>
<th>^Spearman</th>
<th>bρ</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 eMR vs. iMR lower</td>
<td>0.022</td>
<td>* -0.29</td>
<td>115</td>
</tr>
<tr>
<td>A1 eMR vs. iMR upper</td>
<td>0.012</td>
<td>* -0.25</td>
<td>108</td>
</tr>
<tr>
<td>PAF eMR vs. iMR lower</td>
<td>0.043</td>
<td>** -0.61</td>
<td>41</td>
</tr>
<tr>
<td>PAF eMR vs. iMR upper</td>
<td>0.709</td>
<td>-0.26</td>
<td>38</td>
</tr>
<tr>
<td>A1+PAF eMR vs. iMR lower</td>
<td>&lt; 0.0005</td>
<td>** -0.33</td>
<td>156</td>
</tr>
<tr>
<td>A1+PAF eMR vs. iMR upper</td>
<td>0.005</td>
<td>* -0.22</td>
<td>146</td>
</tr>
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</table>

^p value for Spearman Test
bρ for Spearman test
call results were similar for iSR
Table 4: Relationship of intensity tuning of lower and upper suppressive bands

<table>
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<tr>
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<th>Spearman</th>
<th>Linear regression</th>
<th>N</th>
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<tr>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1 IMR</td>
<td>0.36</td>
<td>0.001 **</td>
<td>0.39</td>
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<tr>
<td>PAF IMR</td>
<td>0.47</td>
<td>0.016 *</td>
<td>0.42</td>
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* Similar results were obtained for iSR
Table 5: Relationship of the inhibitory domain to excitatory domain intensity tuning

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<th>Abutting</th>
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<tbody>
<tr>
<td></td>
<td>p</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>IS$_{5-45}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBUE A1</td>
<td>.017 *</td>
<td>-.245</td>
<td>Yes 95</td>
</tr>
<tr>
<td>UBLE A1</td>
<td>.035 *</td>
<td>.235</td>
<td>Yes 82</td>
</tr>
<tr>
<td>LBLE A1</td>
<td>.760</td>
<td>.030</td>
<td>No 95</td>
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<tr>
<td>UBU A1</td>
<td>.690</td>
<td>.050</td>
<td>No 82</td>
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<tr>
<td>IS$_{5-45}$</td>
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<tr>
<td>LBUE PAF</td>
<td>.007 **</td>
<td>-.429</td>
<td>yes 41</td>
</tr>
<tr>
<td>UBLE PAF</td>
<td>.018 *</td>
<td>.391</td>
<td>yes 38</td>
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<tr>
<td>LBLE PAF</td>
<td>.471</td>
<td>-.114</td>
<td>no 41</td>
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<tr>
<td>UBE PAF</td>
<td>.926</td>
<td>-.092</td>
<td>no 38</td>
</tr>
<tr>
<td>CF$_{LB-CF_E}$ A1</td>
<td>.503</td>
<td>-.058</td>
<td>135</td>
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<td>CF$_{UB-CF_E}$ A1</td>
<td>.253</td>
<td>.101</td>
<td>130</td>
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<tr>
<td>UBE A1</td>
<td>.006 **</td>
<td>.300</td>
<td>yes 82</td>
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<tr>
<td>LBUE A1</td>
<td>.036 *</td>
<td>-.216</td>
<td>yes 95</td>
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<tr>
<td>CF$_{LB-CF_E}$ PAF</td>
<td>.929</td>
<td>-.014</td>
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<td>CF$_{UB-CF_E}$ PAF</td>
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<td>-.037</td>
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<tr>
<td>UBE PAF</td>
<td>.033 *</td>
<td>.350</td>
<td>yes 38</td>
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<tr>
<td>LBUE PAF</td>
<td>.006 **</td>
<td>-.438</td>
<td>yes 41</td>
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Figures

**Legend**
- ● inhibitory synapse (input)
- ▲ excitatory synapse (input)
- ▲ suppressive domain
- ▼ excitatory domain

**A.** Intensity-tuned pathway - broad inhibitory inputs - inhibition is strong

**B.** Intensity-tuned pathway - broad inhibitory inputs - inhibition is weak

**C.** Intensity-untuned pathway - narrow 'U' shaped inputs

Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig 6
A. Excitatory & Inhibitory Inputs

B. Excitatory & Suppressive Domains

Lower band
Lower edge (LBLUE)
Upper band
Upper edge (UBUE)

C. IS\textsubscript{S-45}

Fig. 7
Fig. 8
Fig. 9
A. ED-Intensity-tuned pathway - broad inhibitory inputs - inhibition is strong

Level 1 → Level 2

Level 3 inhibition

Level 3 inhibition

Level 3 inhibition

Level 3 inhibition

Level 3 inhibition

Level 3 inhibition

Legend:
- inhibitory synapse
- excitatory synapse
- simultaneous 2-tone inhibitory (TTI) domain
- forward masked 2-tone inhibitory (TTI) domain
- excitatory domain

B. ED-Intensity-untuned pathway - broad inhibitory inputs - inhibition is weak

Level 1

Level 2

Level 3

C. ED-Intensity-untuned pathway - narrow "U" shaped inputs

Level 3 neurons' simultaneous TTI intensity tuned

Level 3 neurons' simultaneous TTI intensity tuned

Level 3 neurons' simultaneous TTI intensity tuned

Level 3 neurons' simultaneous TTI intensity tuned

D. Schematic of Figure 5B

Schematic for Calford & Semple

Fig. 11