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# OVER-REPRESENTATION OF SPECIES-SPECIFIC VOCALIZATIONS IN THE AWAKE MOUSE INFERIOR COLLICULUS

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**Abstract—Social vocalizations are particularly important stimuli in an animal's auditory environment. Because of their importance, vocalizations should be strongly represented in auditory pathways. Mice commonly emit ultrasonic vocalizations with spectral content between 45 and 100 kHz. However, there is limited representation of these ultra-high frequencies (particularly those greater than 60 kHz) throughout the ascending auditory system. Here, we show that neurons in the inferior colliculus (IC) of mice respond strongly to conspecific vocalizations even though the energy in the vocalizations is above the neurons' frequency tuning curves. This results in an over-representation of species-specific vocalizations in the IC. In addition, neurons in mouse IC show selectivity among different vocalizations. Many vocalization-responsive neurons do not respond to the individual ultrasonic frequencies contained within the vocalizations, but they do respond to combinations of ultrasonic tones if the difference between the tones is within the excitatory frequency tuning curve. The combinations of tones that elicit responses are the quadratic and/or cubic intermodulation distortion components that are generated by the cochlea. Thus, the intermodulation distortions in the cochlea may provide a previously overlooked mechanism for auditory processing of complex stimuli such as vocalizations. The implication of these findings is that nonlinear interactions of frequencies, possibly caused by distortions in the system, may be used to enhance the sensitivity to behaviorally important stimuli. Published by Elsevier Ltd on behalf of IBRO.**

**Key words: hearing, frequency tuning, ultrasonic, neural coding, complex sounds, cochlear distortion.**

Two fundamental functions of sensory systems are to detect behaviorally relevant signals in complex environments and to discriminate between these signals so appropriate motor behaviors can be performed. For the auditory system, vocalizations represent one type of behaviorally relevant stimuli. Species-specific vocalizations are used by many different animals to facilitate important behaviors such as mating, territorial defense, and parent–offspring interactions, with different vocalizations conveying different information. The hearing sensitivity of many animals corresponds to the frequency range utilized in vocalizations suggesting that auditory systems have evolved to enhance detection of behaviorally relevant signals (Casseday and Covey, 1996).

Interestingly, the mouse auditory system does not seem to fit this pattern, at least based on stimulating neurons with pure tone stimuli. Both male and female mice emit a variety of ultra-high frequency vocalizations (UHFVs) with spectral content between 45 and 100 kHz (Holy and Guo, 2005; Houseknecht, 1968; Portfors, 2007; Smith, 1975). Males emit UHFVs during inspection and mounting of females, and different vocalization types are correlated with copulation behaviors (Whitney et al., 1973; Wang et al., 2008). These male-emitted copulation UHFVs have behavioral importance for the female as demonstrated by females spending more time with vocalizing males than males who have been surgically devocalized (Pomerantz et al., 1983). Female mice also emit UHFVs, although in different behavioral contexts than males. Females vocalize in the presence of other females and, in particular in response to a female that is reuniting with her group-mates (D'Amato and Moles, 2001; Moles et al., 2007; Wang et al., 2008). In general, female mice use UHFVs in social investigation contexts whereas males use UHFVs to facilitate mating. Although the behavioral meaning and context of the UHFVs varies across vocalization type and gender of emitter (and likely other variables), there is little doubt that UHFVs are behaviorally relevant to mice.

It is surprising then that there is limited representation of the ultra-high frequencies used in vocalizations (particularly those greater than 60 kHz) throughout the mouse ascending auditory system (Liu and Schreiner, 2007; Muller et al., 2005; Portfors and Felix, 2005; Romand and Ehret, 1990; Stiebler and Ehret, 1985; Stiebler et al., 1997). For example, in the main auditory midbrain nucleus, the inferior colliculus (IC), the representation of different frequency ranges is not equal with frequencies between 15 and 26 kHz over-represented (occupying 56% of the whole IC volume) and frequencies above 45 kHz under-represented (Romand and Ehret, 1990; Stiebler and Ehret, 1985). Frequencies above 45 kHz are found in the most medial regions of the IC, often outside of the central nucleus of the IC, and occupy a small percentage of the total volume of the IC (Stiebler and Ehret, 1985). In most studies of IC, neurons with best frequencies greater than 60 kHz are rarely found and lowest tone thresholds for frequencies around 60 kHz are at least 40–50 dB higher than thresholds to neurons tuned to 15–30 kHz (Hage and Ehret, 2003; Portfors and Felix, 2005; Romand and Ehret, 1990; Stiebler and Ehret, 1985). In addition, frequency tuning curves rarely extend beyond 60–70 kHz. These electrophysiological data are congruent with behavioral

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*Abbreviations:* CF, characteristic frequency; FFT, fast Fourier transform; IC, inferior colliculus; MT, minimal threshold; PSTH, post-stimulus time histogram; SI, selectivity index; UHFV, ultra-high frequency vocalization.

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thresholds to tones in the frequency range commonly used in mouse vocalizations (Ehret, 1974). Behavioral responses to pure tones can be obtained up to about 90 kHz, but the thresholds to these ultra-high frequencies are at least 80 dB SPL. Thus, it is unclear how the UHFVs of mice are encoded by the auditory system.

In this study, we first examined whether neurons in the IC of awake mice can detect vocalizations with spectral content outside the frequency range of most neuronal excitatory frequency tuning curves. We then examined whether neurons in the IC of mouse show selectivity among vocalizations to determine whether the IC is capable of discriminating among different vocalizations. We focused on the IC because it receives convergent excitatory and inhibitory inputs from most lower brainstem structures (Brunso-Bechtold et al., 1981; Casseday et al., 2002; Malmierca, 2005) resulting in the creation of novel response properties. In particular, studies in bats provide evidence that the IC is the first site in the ascending auditory system where individual neurons show selectivity among vocalizations; each neuron responds to only a subset of vocalizations even though non-eliciting vocalizations have energy within the excitatory frequency tuning curve of the neuron (Holmstrom et al., 2007; Klug et al., 2002; Portfors, 2004; Xie et al., 2005). The level of selectivity across neurons varies, with some neurons responding to only one vocalization and others responding to many vocalizations but often with different temporal firing patterns. Selectivity to particular vocalizations is sometimes due to inhibition surrounding the excitatory tuning curve (Klug et al., 2002; Xie et al., 2005), inhibition far from the excitatory tuning curve (Holmstrom et al., 2007) or nonlinear facilitation between multiple harmonics in the vocalization (Portfors, 2004).

Each of the studied neural mechanisms underlying selectivity to vocalizations requires that the response-eliciting vocalizations have some match between their spectral content and the frequency tuning curve of the neuron. One way that neurons could respond to vocalizations that have spectral content far outside the neurons' frequency tuning curve is to take advantage of nonlinearities in the transduction of sound to the auditory nerve. Recent evidence shows that IC neurons respond to cochlear distortions generated by combinations of pure tones that individually do not evoke responses (Abel and Kossl, 2009). Because mice emit ultra-high frequencies in their behaviorally relevant vocalizations, in this study we examined whether IC neurons respond to cochlear distortions generated by combinations of ultra-high frequencies. We found that neurons in IC do respond to cochlear distortions and this may be a mechanism utilized for encoding behaviorally relevant complex sounds such as conspecific vocalizations.

## EXPERIMENTAL PROCEDURES

### Surgical procedures

Female CBA/CaJ mice were used in this study. This strain exhibits normal hearing sensitivity well into its second year of life (Willott, 1986, 1991, 2005). The care and experimental manipulations of the animals were carried out in accordance with guidelines of the National Institutes of Health and have been approved by the Washington State University Institutional Animal Care and Use Committee. Number of animals used and their suffering was minimized.

To enable extracellular recordings in the awake mouse, we cemented a metal pin onto the skull of the animal that was later used to secure the head into the stereotax (Portfors and Felix, 2005; Felix and Portfors, 2007; Portfors and Roberts, 2007). The surgery was done one or two days prior to extracellular recordings. The animal was anesthetized with isoflurane inhalation and placed in a rodent stereotaxic frame with a mouse adaptor. A midline incision was made in the scalp and the skin reflected laterally. A tungsten ground electrode was cemented into the right cerebral cortex and the metal pin was cemented onto the skull using ultraviolet-cured dental cement. Using stereotaxic coordinates (Paxinos and Franklin, 2001) and surface landmarks, a craniotomy was made over the IC. A local anesthetic (lidocaine) and topical antibiotic (neosporin) were applied to the wound. The animal was returned to its home cage to recover from surgery prior to starting electrophysiological recordings.

### Acoustic stimulation

Pure tone stimuli and natural mouse social vocalizations were presented as stimuli. Pure tone stimuli were synthesized using custom-written C++ computer algorithms. The pure tone stimuli were 50–100 ms duration, had 1 ms rise/fall times and were presented at a rate of four/s. The social vocalizations consisted of a suite of 16 calls. These vocalizations were recorded from CBA/CaJ adult mice in our laboratory and are known to be regularly emitted by mice living in captivity (Portfors, 2007). The vocalizations do not represent the entire repertoire of mouse vocalizations, but rather represent a variety of commonly emitted ultrasonic calls and two low frequency calls that both males and females emit. The amplitude of the vocalizations was adjusted so that they were all output at the same peak intensity. All vocalization stimuli were stored in the computer prior to electrophysiological recordings. All sound stimuli were output through a high-speed, 16-bit digital-to-analog converter (Microstar Laboratories, Bellevue, WA; 400,000 samples/s), fed to a programmable attenuator (Tucker Davis Technologies, Alachua, FL; PA5), a power amplifier (Parasound) and to a leaf tweeter speaker (Emit) located 10 cm away from the mouse. The acoustic properties of the system were regularly tested using a 1/4 in. calibrated microphone (Bruel and Kjaer, Denmark; model 4135) placed in the position normally occupied by the animal's ear. For pure tone stimuli, there was a smooth, gradual decrease in sound pressure from 6 to 100 kHz of about 2.7 dB per 10 kHz. Distortion components in tonal stimuli were buried in the noise floor, at least 50 dB below the signal level, as measured by custom-designed software performing a fast Fourier transform (FFT) of the digitized microphone signal. To determine whether responses to combinations of signals with frequencies above 45 kHz were due to speaker distortion, we also examined the FFT of each high frequency tone pairs. No speaker distortion was present in any of the two-tone combination stimuli. Vocalization stimuli were calibrated as described above for pure tone stimuli. Potential distortions in the vocalizations were examined by performing a FFT of each digitized vocalization. Low frequency noise in any UHFVs was eliminated prior to output by the speaker by passing the signal through a high-pass filter with a cutoff of 20 kHz. No noise or distortions were present in the vocalization stimuli.

### Extracellular recording procedure

The mouse was briefly sedated with acepromazine and restrained in a piece of foam molded to its body, and the pin attached to its head was secured to a bar on a custom-designed stereotaxic apparatus that was housed in a single-walled sound-attenuating chamber. To obtain well-isolated single unit responses, we used micropipettes filled with 1 M NaCl (resistances of 20–30 MΩ). Electrodes were advanced into the IC by a hydraulic microposi-

tioner (David Kopf Instruments, Tujunga, CA) located outside the acoustic chamber. Electrode penetrations were dorsal–ventral to record from neurons throughout the central nucleus of the IC. Extracellular action potentials were amplified (Dagan Corporation, Minneapolis, MN), filtered (bandpass, 500–6000 Hz; Krohn-Hite, Brockton, MA) and sent through a spike enhancer (Fredrick Haer, Bowdoin, ME) before being digitized (Microstar Laboratories, Bellevue, WA; 10,000 samples/s). Individual neural waveforms were displayed and archived using custom-written C++ software. The software displayed raster plots, post-stimulus time histograms (PSTHs), and statistics on-line. Spike discrimination, spike enhancement, and time-window analysis parameters could be altered offline to analyze stored raw waveforms. Raster and PSTH data were further analyzed and displayed using custom written MATLAB (The MathWorks, Inc., Natick, MA) programs. Each recording session lasted 6–8 h and one to three sessions were conducted on each animal. Petroleum jelly was used to protect the exposed brain between recording sessions. If the animal struggled during experiments, it was removed for the day and recordings were resumed on a subsequent day.

### Stimulus protocol

Pure tones (100 ms duration) were used as search stimuli. Once a single unit was isolated, characteristic frequency (CF) and minimal threshold (MT) were determined audiovisually and later confirmed with quantitative frequency tuning tests. The CF was defined as the frequency at which a unit evoked spikes to at least 50% of the stimulus presentations at MT, and MT was defined as the minimum threshold required to evoke a response to 50% of the stimuli at the CF.

### Single tone stimuli

To obtain excitatory frequency tuning curves, we presented pure tone bursts (100 ms duration, 1 ms rise/fall time, four/s, 200 ms recording window) across the majority of the mouse hearing range (6–80 kHz in 2 kHz steps) in 20 dB intensity steps from 10 dB above threshold to approximately 80–90 dB SPL. Each frequency–intensity pair was presented 20 times. Twenty repetitions without a stimulus were used to calculate spontaneous rate.

### Combination stimuli with one tone set at CF

Because we previously showed that combination-sensitive inhibition (Holmstrom et al., 2007) and facilitation (Portfors, 2004) are mechanisms utilized by IC neurons in the mustached bat to discriminate among conspecific vocalizations, we presented pairs of tones (100 ms duration, 1 ms rise/fall time, 200 ms recording window, simultaneous onset) to test whether similar mechanisms are utilized by neurons in mouse IC. One tone was set at CF and 10–30 dB above threshold such that a consistent spike rate was evoked while the frequency of a second tone varied from 6 to 100 kHz at 20 dB attenuation. Twenty repetitions of each tone pair were presented. If there was evidence of inhibition and/or facilitation, the intensity of the second tone was varied in steps of 10 dB until the inhibition or facilitation was eliminated. This generated a threshold of inhibition or facilitation. Inhibition and facilitation were defined by the spike rate decreasing or increasing by greater than 20% of the sum of the spike rates to the individual tones (Portfors and Wenstrup, 1999; Portfors and Felix, 2005). Inhibition that surrounded the excitatory frequency tuning curves was used to help categorize the neurons into frequency tuning types previously defined for mouse IC (Egorova et al., 2001).

### Ultrasonic tone pairs

To determine whether distortion products could be a mechanism utilized by IC neurons to encode complex, ultra-high frequency stimuli, we presented pairs of ultra-high frequency tones that individually did not evoke neural responses. We arbitrarily set one tone at a high frequency used in many vocalizations (usually 70 or 80 kHz) and varied the frequency of the second tone (between 30–100 kHz) so that the difference between the two frequencies would cover the extent of the neuron's frequency tuning curve. The intensity of the tones was set at 20 dB attenuation (60–80 dB SPL depending on the frequency response of the speaker) because distortion products occur with high intensities (Nuttall and Dolan, 1993; Abel and Kossl, 2009). In some cases, we varied the intensities of the tones to determine a threshold for the neural response to the combination stimuli. The two tones had simultaneous onset, 100 ms duration, 1 ms rise/fall times and were presented at four/s with a 200 ms recording window.

### Vocalization stimuli

Responses to a suite of 16 vocalizations were tested at three intensities (40, 60 and 80 dB; intensities at which the vocalizations are naturally emitted). Twenty repetitions of each stimulus, at each intensity were presented. Vocalizations were different durations, but each was presented at four/s with a recording window of 200 ms.

### Data analysis

Spike counts and raw waveforms were stored in the computer during data collection. Raw waveforms were examined offline to ensure only spikes from well-isolated single units were used in the data analysis. Data were exported from the custom-written data collection software and analyzed using programs written in MATLAB. Responses to single tones were used to generate frequency tuning curves. Frequency tuning curves were generated from the pure tone tests using statistical comparisons between evoked responses and spontaneous activity (Holmstrom et al., 2007).

We categorized the excitatory frequency tuning curves in two ways. First, we utilized the classification scheme of Ehret and colleagues (Egorova et al., 2001) derived for mouse IC. Neurons were classified into types I, II, III and IV based on the slopes of the high and low frequency sides of the excitatory tuning curve, presence of inhibitory sidebands and complex response areas. Second, because one goal of this study was to determine whether frequency tuning curves could predict responses to UHFVs, we developed a classification scheme to distinguish between low and high frequency tuned neurons. Our distinction between low and high frequencies was based on the spectral content of the UHFVs such that low frequency tuning curves were entirely outside the frequency range of the UHFVs. We categorized the frequency tuning curves into four types based on CF and the width of the tuning curve. The four categories were (1) low frequency, narrow; (2) low frequency, broad; (3) high frequency; (4) multiply tuned.

To sort the tuning curves into the four categories, we analyzed the peak responses of an intensity compressed form of the frequency tuning curve, F ($f$, $a$), where $f$ is the frequency (Hz) and $a$ is the SPL intensity (dB). For the intensity compressed form, $F_c(f)$, the tuning curve was summed over the sampled intensities, $F_c(f)=\sum_a F(f,a)$, to yield a profile of the frequency response. We then determined the range of frequencies with responses greater than 0.3 times the maximum, $\{f: F_c(f)>0.3\cdot\max(F_c(f))\}$. If the *maximum f* in this set was *less* than 35 kHz, and the difference between the minimum $f$ and maximum $f$ was *less* than 8 kHz, then the frequency tuning curve was in the low frequency, narrow category. If the *maximum f* was *less* than 35 kHz, and the difference between the minimum $f$ and maximum $f$ was greater than 8 kHz, then the frequency tuning curve was in the low frequency, broad category. If the *minimum f* was greater than 35 kHz, then the frequency tuning curve was in the high frequency category. If there were frequencies in the set that were both greater and less than 35 kHz, then the frequency tuning curve was in the multiply

tuned category. The threshold of 35 kHz was chosen to be reliably lower than the UHFVs. Thus, we predicted that neurons in the low frequency, narrow and low frequency, broad categories would not respond to any of the UHFVs.

For each neuron that responded to at least one vocalization, a selectivity index (SI) was calculated as SI=(*Ct*–*Ce*)/*Ct* where *Ct* is the number of calls presented and *Ce* is the number of calls that evoked a response (at 30–50 dB above threshold). High index values indicated high selectivity.

To determine whether neural responses to combinations of ultra-high frequency tones could be explained by distortions generated in the cochlea, we compared outputs of response models without (linear) and with (nonlinear) a cochlear amplifier. We analyzed neurons where both the frequency tuning curve and ultrasonic combination tone responses were measured (44 neurons). First, we used the excitatory frequency tuning curve, F (*f*, *a*) of each neuron to predict the response evoked by each pure tone frequency–intensity pair (*f*, *a*) or each combination of frequencies and intensities (*f1*, *f2*, *a1*, *a2*). The responses from ultrasonic tone combinations were estimated by adding the nearest measured value from the frequency tuning curve to the frequency–intensity combination.

Using our experimental ultrasonic tone combinations, the model synthesized the signal and estimated the expected response given the response curve. For instance, for each tone and intensity, the nearest measured response was assigned, and multiple tones were summed to yield a linear prediction of the neuron's response.

The tone combinations were synthesized, $x(t)$, and used to predict the expected response from the excitatory frequency tuning curve by computing the power spectral density and converting to intensity level (dB) from our synthesized signal. The sum of each frequency's contribution was modified by a scaling factor and a threshold to control contributions from spontaneous activity. The result was a *linear prediction* of how each neuron would respond to a synthesized tone.

To predict the nonlinear response caused by cochlear distortions, we filtered our synthesized signal by a cochlear model previously used to study intermodulation distortion by the cochlear amplifier (Lukashkin and Russell, 1998):

$$v(x(t)) = \frac{E_e + E_b}{1 + R_a(x)/R_b} - E_b + 74.64$$

$$R_a(x) = R_c + (1 + e^{a_2(x_2 - x_s - y)}(1 + e^{a_1(x_1 - x_s - y)})), \qquad (1)$$

where the parameters were $a_1$=0.065 (1/nm), $a_2$=0.016 (1/nm), $x_1$=24 (nm), $x_2$=41 (nm), $R_b$=50 (MΩ), $R_c$=500 (MΩ), $E_b$=90 (mV), $E_e$=80 (mV), and $x_s$=26 (nm). The output of the filter, $v(x(t))$, contained the distortion products that were resolved by computing the power spectral density and converting to intensity level (dB). Using the frequency tuning curve as above, we could derive a nonlinear, *Lukashkin prediction* of each neuron's response.

In the ultrasonic combination tone experiment, one tone was presented with a fixed frequency (*f1*) while the frequency of a second tone (*f2*) was varied. The response recorded for each tone pair presentation was $r(f2)$. The linear model prediction, $\hat{r}_l(f2)$, and the nonlinear Lukashkin model prediction, $\hat{r}_n(f2)$, were compared with the recorded response using a mean square error, $M_l = \sum_{f2}(r(f2) - \hat{r}_1(f2))^2/N$, and $M_n = \sum_{f2}(r(f2) - \hat{r}_n(f2))^2/N$, where $N$ is the number of frequency steps. The scaling factor and a threshold of the model for each neuron were optimized to minimize $M_n$, and the same parameter setting were used for $M_l$. The relative difference between the linear and nonlinear models, $D = (M_n - M_l)/(M_n + M_l)$ was then calculated to determine whether the Lukashkin filter improved the prediction, where positive values imply a better prediction by the nonlinear model, and negative values imply a better prediction by the linear model.

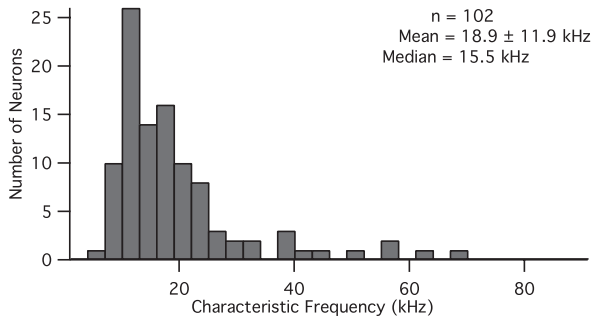


**Fig. 1.** Characteristic frequencies of IC neurons.

## RESULTS

We recorded well-isolated single unit responses to vocalizations in 102 neurons. These neurons had a range of characteristic frequencies between 6 and 68 kHz with the majority of CFs below 30 kHz (Fig. 1). Seventy-nine neurons responded to at least one of the vocalizations at one or more intensities. Because the focus of this study was on how IC neurons detect and discriminate vocalizations rather than other general properties of IC neurons, only those neurons that responded to at least one vocalization are included in the rest of the Results section.

We classified the frequency tuning curves of the vocalization-responsive neurons in two ways. First, we utilized the classification scheme derived by Ehret and colleagues (Egorova et al., 2001) for IC neurons and found representatives in each of the classes (types I, II, III and IV). The proportions of neurons in each class were consistent with our previous study in the awake CBA/CaJ mouse (Portfors and Felix, 2005). Second, because one goal of this study was to determine whether frequency tuning curves could predict responses to UHFVs, we developed a classification to distinguish between low and high frequency tuned neurons. Fig. 2 shows an example of each of the four frequency tuning categories. (A) *Low frequency, narrow* tuning curves (Fig. 2A) were found in 34% of the neurons (27/79). These neurons responded to pure tone frequencies below the range of high frequency vocalizations. (B) *Low frequency, broad* tuning curves (Fig. 2B) were found in 23% of the neurons (18/79). These neurons had broad tuning defined by our criteria (see Experimental Procedures), but no sensitivity to pure tones at frequencies in the range of most UHFVs (vocalizations with all of their spectral content above 45 kHz.) (C) *High frequency* tuning curves (Fig. 2C) were found in only 4% of the neurons (three/79). The pure tone responses of these neurons predict that they are specialized for encoding the behaviorally significant UHFVs, yet the population of these types of neurons appears to be small. (D) *Multiply tuned* frequency tuning curves (Fig. 2D), with responses in both the low and high frequency ranges, were found in 39% of the neurons (31/79).

Examining the frequency tuning characteristics of all the neurons that responded to vocalizations showed that more than half of the neurons responded to UHFVs even though they did not respond to pure tones in the same

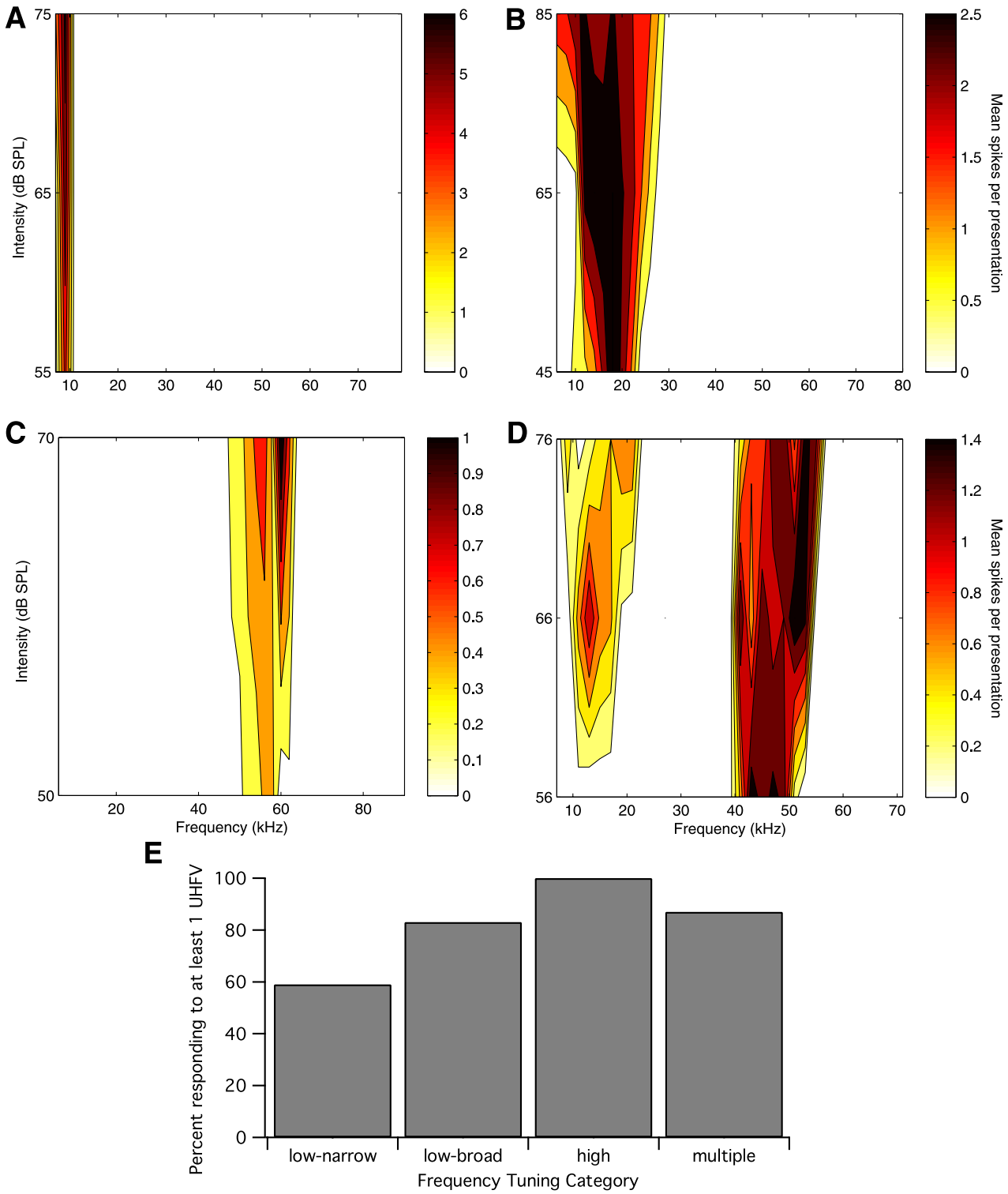


**Fig. 2.** Examples of excitatory tuning curve categories used in this study and percentage of neurons within each tuning class that responded to UHFVs. (A) Low frequency, narrow. The *maximum* frequency in these tuning curves was *less* than 35 kHz and the difference between the minimum and maximum frequencies was *less* than 8 kHz. (B) Low frequency, broad. The *maximum* frequency was *less* than 35 kHz and the difference between the minimum and maximum frequencies was greater than 8 kHz. (C) High frequency. The *minimum* frequency was greater than 35 kHz. (D) Multiply-tuned. There were frequencies both greater and less than 35 kHz. (E) Percentage of neurons in each category that responded to at least one UHFV (all spectral content above 45 kHz).

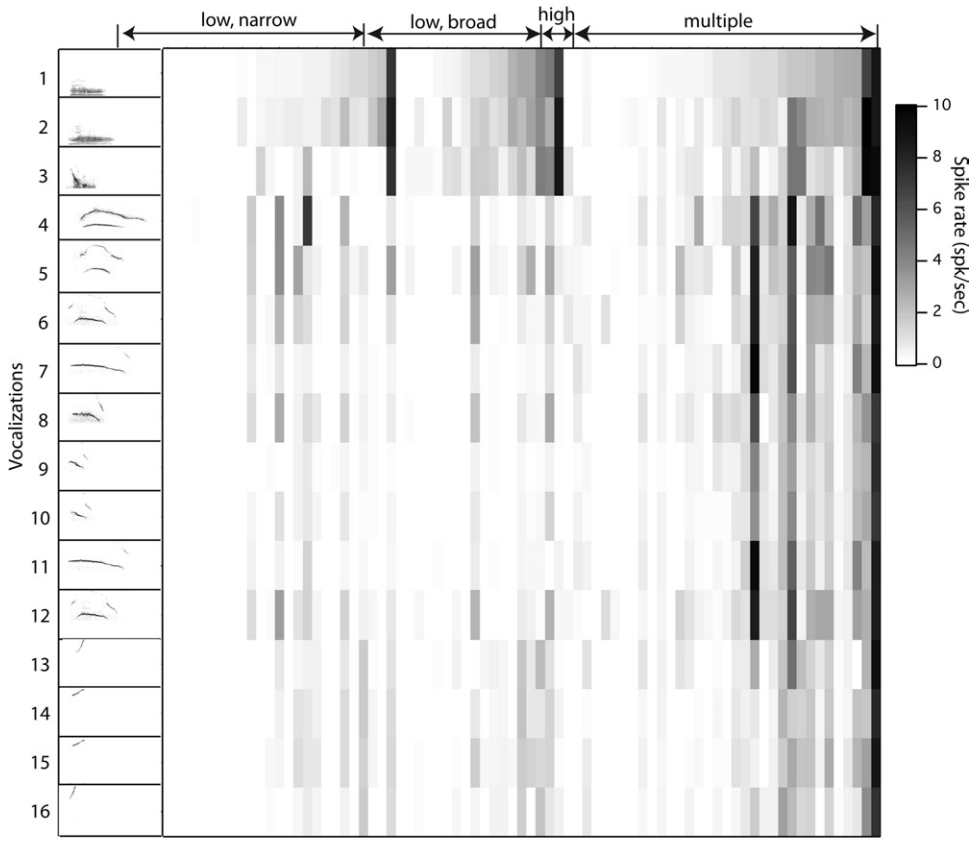


**Fig. 3.** Responses of our sample of 79 neurons to the suite of 16 vocalization stimuli demonstrate selectivity in the IC. The gray-scale shows how strongly each neuron responded to each vocalization (spike rate), revealing a distributed population coding by neurons in the IC. The neurons are grouped by their frequency tuning curve classes across the x-axis. The vocalization stimuli are ordered down the y-axis in ascending order based on the lowest frequency found in each stimulus. The UHFVs are numbered 5–16 (scale: abscissa, 0–200 ms; ordinate, 1–100 kHz). The responses demonstrate that many low-tuned neurons respond to UHFVs with spectral content higher than 45 kHz.

frequency range of the UHFVs (above 45 kHz) (Fig. 2E). In the low frequency tuning classes, 31 of the 45 neurons responded to the UHFVs (16 low, narrow and 15 low, broad). This finding was unexpected as the UHFVs did not contain energy in the excitatory frequency tuning curves of the neurons. It is important to note that these low frequency neurons responded to the UHFVs even when the signals were high-pass filtered at a cutoff of 20 kHz. All of the high frequency neurons responded to UHFVs and 27 of the 31 multiply tuned neurons responded to UHFVs (Fig. 2E).

The majority of neurons had some inhibitory sidebands as has been documented previously in CBA/CaJ IC (Portfors and Felix, 2005). None of the neurons had combination-sensitive responses within the frequency ranges of the ultrasonic vocalizations. Thus, combination-sensitive inhibition and facilitation as tested in this study (with simultaneously presented tone combinations) could not explain any of the responses to UHFVs.

### Neural responses to social vocalizations

To examine the pattern of responses to the suite of vocalizations in all our recorded neurons, we grouped the neurons within their frequency tuning category and plotted each neuronal response (spike rate along the x-axis) to each vocalization (spectrograms displayed down the y-axis according to increasing fundamental frequency) (Fig. 3). The first two vocalizations were low frequency, multiharmonic calls with a fundamental frequency of about 6 kHz. The 3rd and 4th vocalizations had ultrasonic elements but much of the energy was below 45 kHz and thus within the range of many frequency tuning curves. The remainder of the vocalizations had all their spectral content above 45 kHz (UHFVs). Fig. 3 illustrates three important characteristics of IC responses to vocalizations. First, as shown in Fig. 2E and discussed above, neurons with low frequency tuning curves responded to UHFVs. Second, each neuron responded differently to the suite of vocalizations; some neurons had high levels of selectivity and others had low levels of selectivity. In each frequency tuning category (low-narrow, low-broad, high, multiple), there are examples of neurons responding to all 16 vocalizations and examples of neurons responding to one to three vocalizations. In some neurons, spike rate varied greatly in response to the different vocalizations. For example, spike rates were often greatest to the two low-frequency, multiharmonic vocalizations plotted at the top of Fig. 3 and lowest to the four high frequency upsweeps plotted at the bottom of the figure.

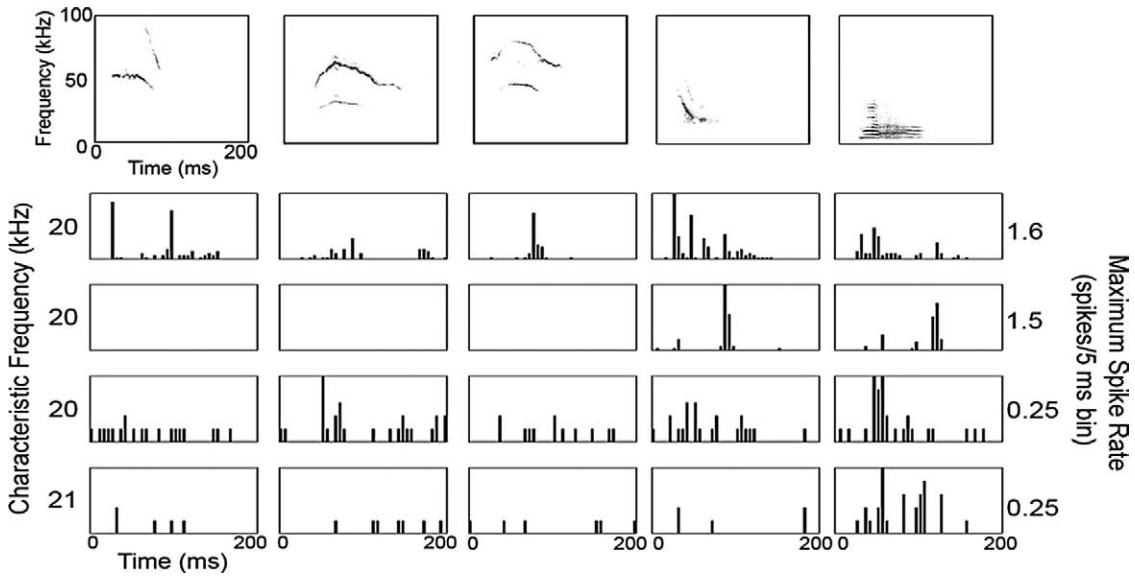


**Fig. 4.** Neurons in mouse IC are selective to vocalizations. Responses to only five of the suite of 16 vocalizations are shown here for clarity. Top row shows spectrograms of the five vocalizations; the first three are UHFVs. Each subsequent row shows the response of one single neuron to the vocalizations. The characteristic frequencies of the four neurons were similar. The first neuron showed no selectivity (it responded to all vocalizations with time-locked spikes) whereas the last neuron showed high selectivity. The last two neurons show spontaneous activity, but were selective in their responses to the stimuli as indicated by time-locked spikes to the vocalizations. Two of the neurons (first and third) responded to the UHFVs even though their excitatory frequency tuning curves did not encompass those frequency bands.

Third, each vocalization elicited a different pattern of activity across the sample of neurons. In examining the population response to one vocalization, neurons with similar CFs and similar tuning curve characteristics (i.e. neurons classified into the same category) showed different responses to one vocalization. The two low frequency, multiharmonic vocalizations elicited the most similar responses from the sample of neurons. The majority of neurons responded to one or both of these vocalizations (see responses to top two vocalizations in Fig. 3) as expected based on the spectral content of the vocalizations and the frequency tuning curves of the neurons. However, spike rates for each of the low frequency vocalizations were different for each neuron. The neural responses to the high frequency, upsweep vocalization plotted at the very bottom of Fig. 3 showed extremely high diversity. Even neurons with similar frequency tuning characteristics responded differently to the same vocalization. Thus, each vocalization activated a unique pattern of responses across the sample of IC neurons.

These three characteristics of neural responses to vocalizations are further illustrated in Fig. 4. Here, we plot PSTHs evoked by five vocalizations (we only plot responses to five vocalizations for clarity) for four neurons with similar CFs and frequency tuning curves. Two of the neurons responded to UHFVs even though their CFs were low frequency (20 kHz). The neuron in the top plot of Fig. 4 responded to all of the UHFV shown (spectrograms 1–3) whereas the neuron in the third row only responded to one of the UHFVs (spectrogram 2). All the neurons displayed in Fig. 4 had CFs and tuning curves below the spectral content of the UHFVs. Thus, the responses to UHFV were not because the energy in the vocalizations fell within the excitatory frequency tuning curve of the neurons. In addition, the neurons had different levels of selectivity; the neuron illustrated in the bottom row responded to only one vocalization (the other spikes are caused by spontaneous activity), whereas the neuron in the top row responded to nine of the 16 vocalizations. In addition, responses to an individual vocalization were different across the four neurons. The response differences were with respect to magnitude and/or temporal firing pattern.

Across the population of recorded neurons, the level of selectivity among the suite of vocalizations differed. Fig. 5 shows the SI values for the 79 neurons that responded to at least one of the 16 presented vocalizations. High index values indicate high selectivity. The highest SI value that could be obtained in our data was 0.94 (response to one of

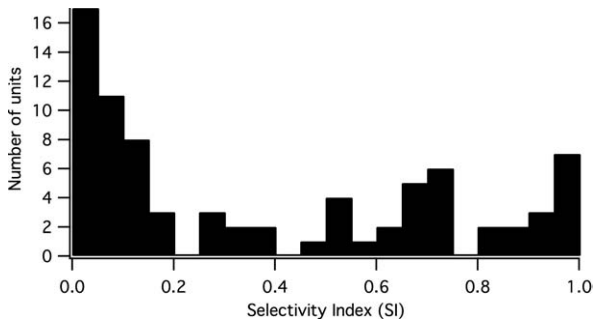


**Fig. 5.** Neurons in mouse IC showed various degrees of selectivity to vocalizations. Histogram of SI values for neurons recorded in awake mouse IC. High index values denote high selectivity. With the suite of 16 vocalization stimuli, the highest SI value that could be generated was 0.94 (the neuron responded to only one of the vocalizations). A value of 0 was obtained if a neuron responded to all the 16 vocalizations.

the 16 calls) and the lowest was 0 (responses to all 16 calls). The mean (SD) SI value was 0.32 (0.30). The majority of neurons responded to many vocalizations (SI<0.2) but as Fig. 5 demonstrates, some IC neurons showed high levels of selectivity among different vocalizations with a few neurons responding to only one to three vocalizations (SI>0.8).

### Neural responses to ultra-high frequency difference tones

One possible mechanism for neurons with low frequency tuning curves to respond to complex signals with spectral content outside their excitatory frequency tuning curve is via nonlinear distortion products generated in the cochlea. We tested 59 neurons for responses to difference tones generated by combinations of ultra-high frequencies. Forty-four neurons responded to some combination of two frequencies, and none of the neurons responded to either signal when presented individually.

Three examples of responses to difference tone combinations are shown in Fig. 6. Each neuron's excitatory frequency tuning curve is also illustrated to help to explain the combination responses. In the first example (Fig. 6A, B), the neuron was multiply-tuned with a CF of 10 kHz and a second excitatory tuning curve around 58 kHz. The neuron responded to both the quadratic (F1–F2, F2–F1) and cubic (2F2–F1, F2–2F1) distortion products (Fig. 6B). When these differences were within the excitatory tuning curve of the neuron, there was a vigorous spiking by the neuron. At other tone combinations, there was little or no evoked response (some spontaneous activity was often present). In addition, the response peaks were greatest for the quadratic distortion product. This is consistent with the magnitude of distortion products generated by a nonlinear filter. However, the cubic distortion product is known to dominate in the otoacoustic emissions (Shera, 2004; Abel and Kossl, 2009). In the mouse IC, we found strong quadratic dominance in many neural responses, as exemplified in Fig. 6D, where the response was only to the quadratic difference tones. In this case, the tuning curve (Fig. 6C) showed a weak response except at high intensities, so that the weaker, cubic distortion products were, presumably, not strong enough to drive the neuron. Other neurons displayed responses where the cubic distortion products dominated as shown in Fig. 6F. Here, the reduced response to the quadratic differences, relative to the cubic differences, can be explained by the "O-type" tuning curve, where there was a reduced response to the highest intensity stimuli when compared with lower stimulus intensities (Fig. 6E). A possible explanation is that the cubic distortion products (2F2–F1, F2–2F1) were strong enough to reach the strongest part of the tuning curve, but the quadratic distortion products (F1–F2, F2–F1) were at a higher intensity, and generated a reduced response.

To determine whether the neural responses to the combinations of ultra-high frequency tones could be explained by distortions generated in the cochlea, we compared responses predicted by a linear model using the excitatory frequency tuning curve and a nonlinear model of the cochlea that contains terms to account for intermodulation distortion (Lukashkin and Russell, 1998) with the empirical responses.

When the distortion products were taken into account, the frequency tuning curve generated by single tones was often found to be a good predictor of the location and width of combination-tone responses. As shown in Fig. 7A and B, the prediction using the Lukashkin filter was similar to the recorded neural responses. However, the Lukashkin filter improved the model predictions in less than half of the neurons tested (19/44). The relative improvement of the prediction was measured by the relative difference between the mean squared error between the predicted response and the recorded response. Some neurons (5/44) showed no difference between the linear model prediction and the nonlinear, Lukashkin model prediction, as shown in the example in Fig. 7C. In a significant number of neurons (20/44), such as the example illustrated in Fig. 7D, the nonlinear model's prediction was worse than the linear model. These neurons generally showed an absence of responses to the combination tones, as if the circuitry between the cochlea and IC reduced or eliminated the effects of cochlear distortions.

### Complex signals also generate responses to difference tones

Having established that combinations of ultrasonic tones evoked responses in IC neurons, we tested whether a more complex signal structure would also evoke responses when there was only a transient combination of tones with distortion products in the frequency tuning curves of the neurons. We combined frequency sweeps so that the individual sweeps would not generate a response, as shown in Fig. 8. The frequency tuning class of this neuron was low, broad tuning (Fig. 8A), and the combination of a 70 kHz tone (F2) with either a 56 kHz tone (F2–F1=14 kHz) or an 84 kHz tone (F1–F2=14 kHz) evoked a response (Fig. 8B). We then synthesized a frequency down-sweep outside of the tuning curve of the neuron, starting at 90 kHz and ending at 70 kHz with a duration of 100 ms (Fig. 8C). We combined the sweep with a constant tone at 85 kHz, and when the difference between the two signals reached 14 kHz, the neuron responded with a latency of 20 ms. We next tested three different sweep rates in combination with the 85 kHz pure tone and a response was consistently evoked 20 ms following the time when the difference between the signals reached 14 kHz. For the trial where there was never a difference of 14 kHz, no response was evoked. Thus, responses to complex sounds such as frequency sweeps in the IC can be explained by combinations of ultra-high frequencies that generate distortion products in the cochlea.

### Non-simultaneous combination tones generate responses in IC

Some responses of low-frequency tuned neurons to UHFVs cannot be explained by combination of tones presented simultaneously. For example, the neuron in Fig. 9

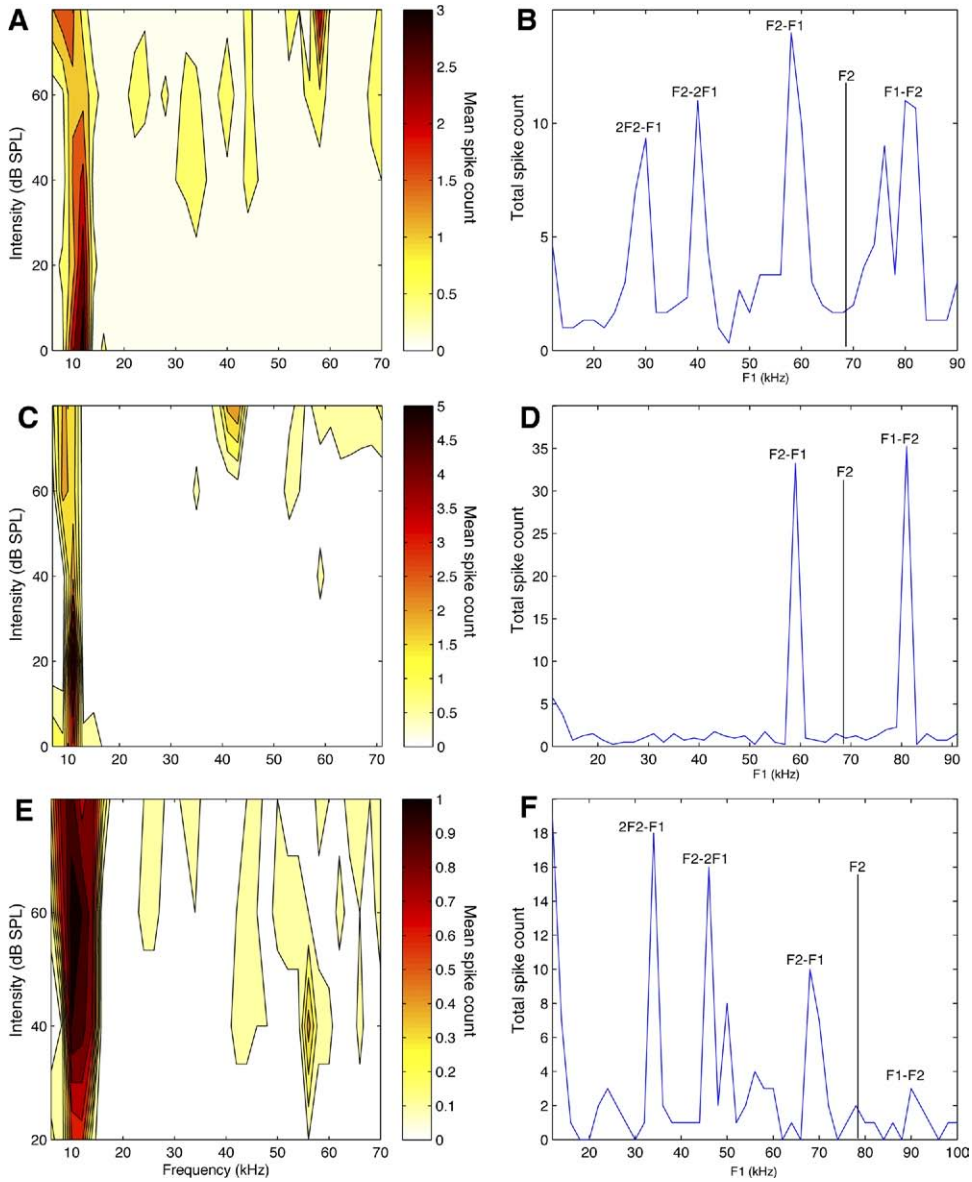


**Fig. 6.** Neurons in IC respond to combinations of different ultra-high frequency tones. Responses of three representative neurons are shown. (A, C, E) The single tone frequency tuning curves for the three neurons. (B, D, F) The neural responses to combinations of ultra-high frequency tones. The frequency of one ultra-high frequency tone remained constant (vertical line designated as F2) and the frequency of a second simultaneously presented tone varied (F1, x-axis). (A, B) The low frequency portion of the tuning curve had a low threshold resulting in the neuron responding to the quadratic (F1–F2, F2–F1) and cubic (2F2–F1, F2–2F1) distortion products. The stronger response to the F2–F1 distortion product may be the result of the summation with the second excitatory tuning curve at 58 kHz. (C, D) This neuron only responded to the quadratic distortion products. (E, F) Responses to the cubic distortion products were more prominent in this neuron. All tones were presented at 20 dB attenuation (60–70) dB SPL depending on F1 and F2 frequencies; see Experimental Procedures for description of speaker output fall-off with increasing intensity.

responded to the vocalization shown in Fig. 9B even though that vocalization did not have simultaneous combinations of frequencies creating differences tones within the neuron's excitatory frequency tuning curve. Several vocalizations in our suite had this basic spectral–temporal structure: a simple frequency modulated upsweep with varying amplitude. While these vocalizations do not have *overlapping* frequencies that produce difference tones within the tuning curve of the responding neurons, they do have frequency *modulations* that may cause resonances on the basilar membrane that overlap. Thus, low-frequency tuned neurons in IC could be responding to these non-simultaneous difference tones resulting in a response to the vocalization.

To test whether frequency differences presented at different times could generate responses in IC, we combined a tone with a frequency sweep that passed through a frequency difference in the neuron's frequency tuning curve (Fig. 9C). We selected this particular combination to simulate one of the natural vocalizations to which the neuron responded. The neuron did not respond to the two components of the synthesized vocalization when pre-

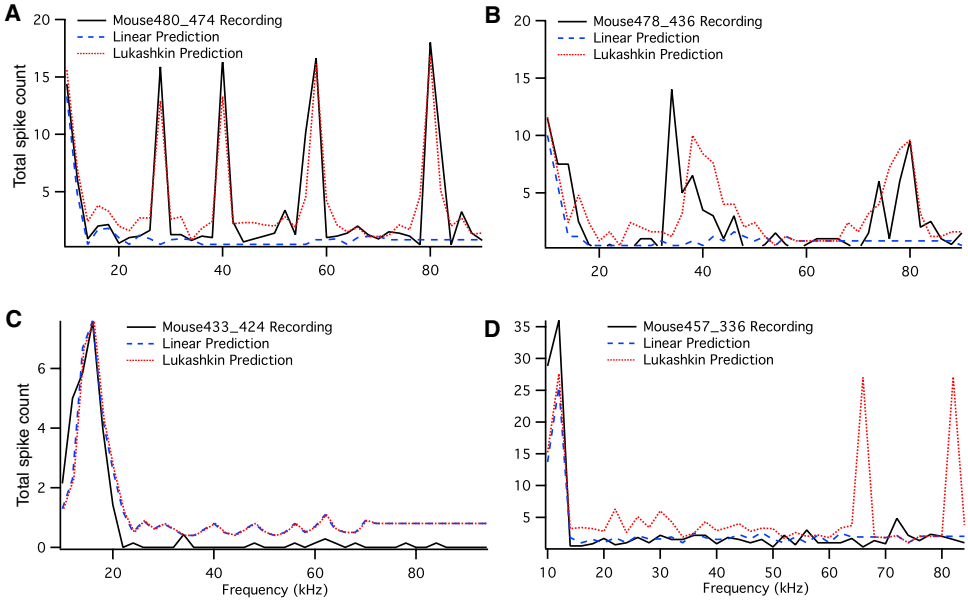


**Fig. 7.** Response model predictions compared with electrophysiological recordings. Responses of four different neurons to combinations of ultrasonic tones are shown and compared to predictions generated by linear and nonlinear response models. (A) This neuron responded to combinations of ultrasonic tones (F2=70 kHz, F1=abscissa). The linear model did not predict distortion products and consequently predicted that the neuron would not respond to combination tones. If the signal was prefiltered with the Lukashkin cochlear amplifier, then the model generated distortion products and consequently predicted the neuron would respond to combination tones. This prediction matched the actual neural responses. (B) In this neuron, the frequency tuning was broad and a broad range of tone combinations evoked responses. The linear filter did not predict neural responses. The Lukashkin cochlear amplifier model correctly predicted the broad response to the combination tones (F2=70 kHz) that resulted from the broad frequency tuning curve. (C) In this neuron, combination tones did not evoke responses, and both the linear and the Lukashkin models correctly predicted a lack of combination tone responses. (D) In this neuron, combination tones did not evoke any responses. The Lukashkin cochlear amplifier model predicted responses to combination tones. The linear model correctly predicted the absence of responses to combination tones, possibly due to the presence of inhibition in the circuit that eliminates the distortion products. All tones were presented at 20 dB attenuation (60–70) dB SPL depending on F1 and F2 frequencies; see Experimental Procedures for description of speaker output fall-off with increasing intensity.

sented separately (Fig. 9C) because the neuron's frequency tuning curve was below the spectral content of the vocalization. When we presented the synthesized signals, a difference tone would only be generated by frequencies that were separated by up to 10 ms, the duration of the frequency sweep. Fig. 9D illustrates that the neuron did respond to the synthesized signal. Thus, the tone differences do not need to be simultaneous.

In addition, this example demonstrates that the relative amplitude of the synthesized segments was critical for the response. The strongest responses were to the combination that most closely resembled the original vocalization. Furthermore, the response was independent of the overall intensity of the synthesized vocalization; the neuron responded to the *relative* difference of the components. The implication of this response feature is that, in a natural situation, the neuron would be selective to the vocalization independent of the distance from the source.

## DISCUSSION

There are three main findings of this study. First, some neurons in mouse IC responded to high frequency vocalizations even though their frequency tuning curves were lower than the frequency range of the vocalizations. Second, neural selectivity among conspecific vocalizations occurs in IC. Third, neurons in IC responded to difference tones created by combinations of ultra-high frequencies.

### Detection and discrimination of vocalizations in IC

In this study we found that the majority of neurons in mouse IC respond to conspecific vocalizations. Not surprisingly, some responses to vocalizations are due to a match between the neuron's excitatory frequency tuning curve and the spectral content of the vocalization. For example, the majority of neurons tuned to frequencies less than 20 kHz responded to the low, frequency multiharmonic vocalizations. In all cases, at least one of the harmonics fell within the excitatory frequency tuning curve of the neuron. Similarly, neurons with single, high frequency tuning curves ($n$=3) or multiple tuning curves with one region in high frequencies ($n$=31) responded to the vocalizations that had matching high frequency content. However, rarely did the high frequency or multiply tuned neurons have excitatory regions that reached the ultra-high frequencies found in many behaviorally relevant vocalizations (Holy and Guo, 2005; Portfors, 2007; Wang et al., 2008). For example, no neurons had tuning curves that extended up to 80 kHz even though the frequency range of four vocalizations was between 80 and 100 kHz. In fact, we found very few neurons that responded to pure tones

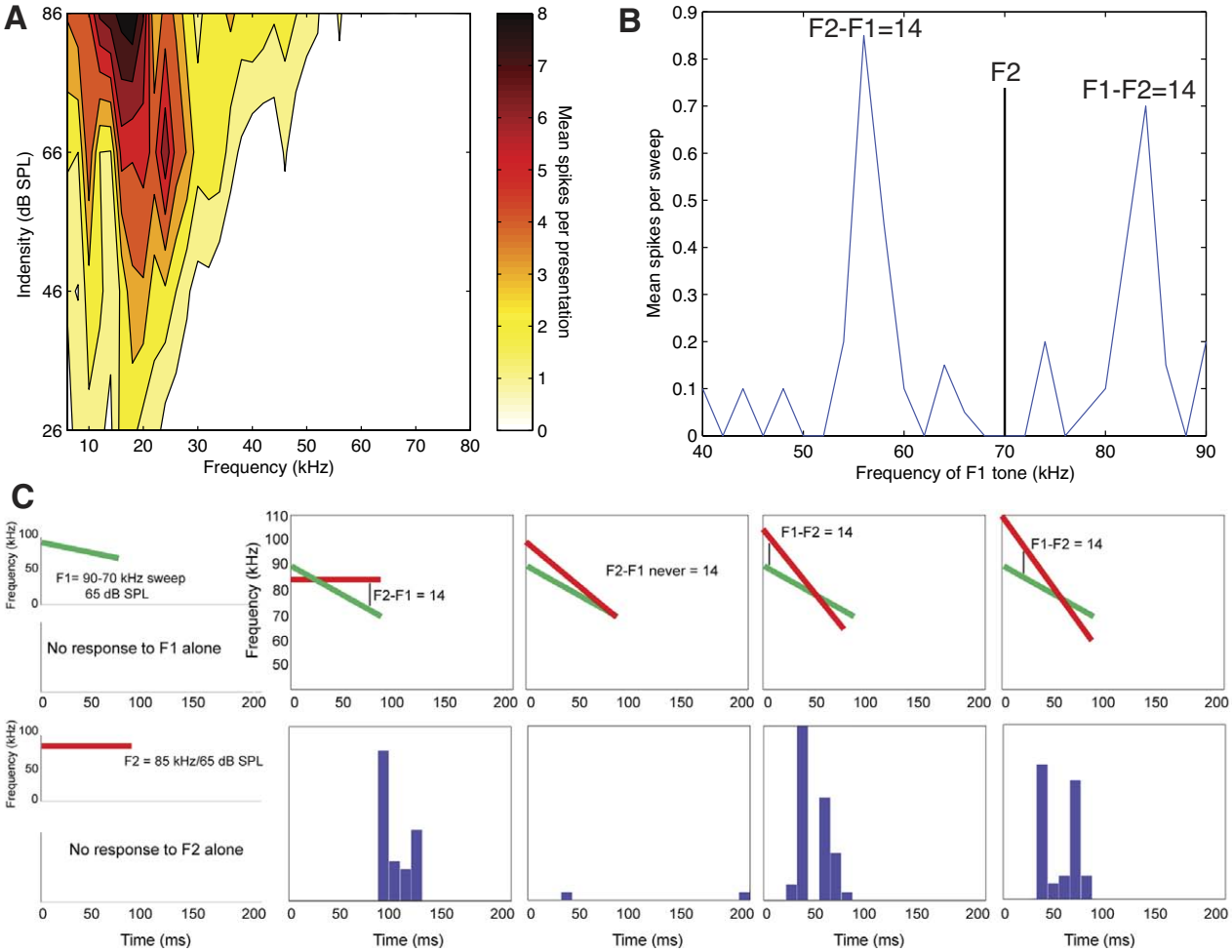


**Fig. 8.** Responses to combinations of ultra-high frequency stimuli occur when the frequency difference between the two stimuli is within the neuron's frequency tuning curve. (A) Frequency tuning curve of a neuron in the low-broad tuning class. (B) Responses to combinations of ultra-high frequency tones. One tone was set at 70 kHz (vertical line at F2) and the second tone varied from 40 to 90 kHz. The neuron did not respond to the individual frequencies but responded when the difference between the two stimuli was within the frequency tuning curve. The neuron responded to the quadratic (F1–F2, F2–F1) distortion products. (C) Combinations of frequency-modulated sweeps show the same results with the addition that the latency of the response is determined by the time at which the combination of the two sweeps creates a quadratic distortion product. The neuron did not respond to either stimulus presented alone. F1 (green line) was set as an FM sweep from 90 to 70 kHz in 100 ms. The parameters of F2 (red line) varied. In the first plot, F2 was a pure tone at 85 kHz (no response to this stimulus alone). In the second plot, F2 was presented as an 85 kHz tone at the same time as F1. A difference of 14 kHz occurred near the end of the stimuli and the response occurred 10 ms later. In the third plot, F2 swept from 95 to 70 kHz. The overlapping stimuli never differed by 14 kHz and the neuron did not respond. In the fourth plot, F2 swept from 105 to 65 kHz. The two stimuli differed by 14 kHz near the start of the stimuli and the latency of the response was short. In the fifth plot, F2 swept from 110 to 60 kHz, a difference of 14 kHz occurred 25 ms into the stimulus and the response was 10 ms later. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

greater than 60 kHz (at intensities up to 80 dB SPL). These results and those from other studies (Hage and Ehret, 2003; Portfors and Felix, 2005; Romand and Ehret, 1990; Stiebler and Ehret, 1985) demonstrate that the mouse auditory system devotes very little space to frequencies contained in many behaviorally relevant vocalizations.

The surprising result of this study is that many neurons that responded to low frequency pure tones also responded to vocalizations that had all spectral content outside the neuron's frequency tuning curve. Our results support previous findings that the way a neuron in IC responds to vocalizations cannot necessarily be predicted based on the neuron's excitatory frequency tuning curve generated by pure tones (Klug et al., 2002; Holmstrom et al., 2007). We classified each neuron's excitatory frequency tuning curve into low frequency (low, narrow and low, broad), high frequency and multiple tuning based on a cutoff of 35 kHz such that neurons with all their excitation below 35 kHz were classified as low tuned. The cutoff of 35 kHz was chosen because this frequency is reliably lower than the spectral content of the mouse ultrasonic vocalizations (Holy and Guo, 2005; Portfors, 2007; Wang et al., 2008). We predicted that the low-tuned neurons would not respond to the vocalizations with ultra-high frequencies ($>$45 kHz). However, we found that almost 70% of the low-tuned neurons did respond to at least one of the UHFVs even though there was no match between the excitatory frequency tuning curve of the neurons and the spectral con-

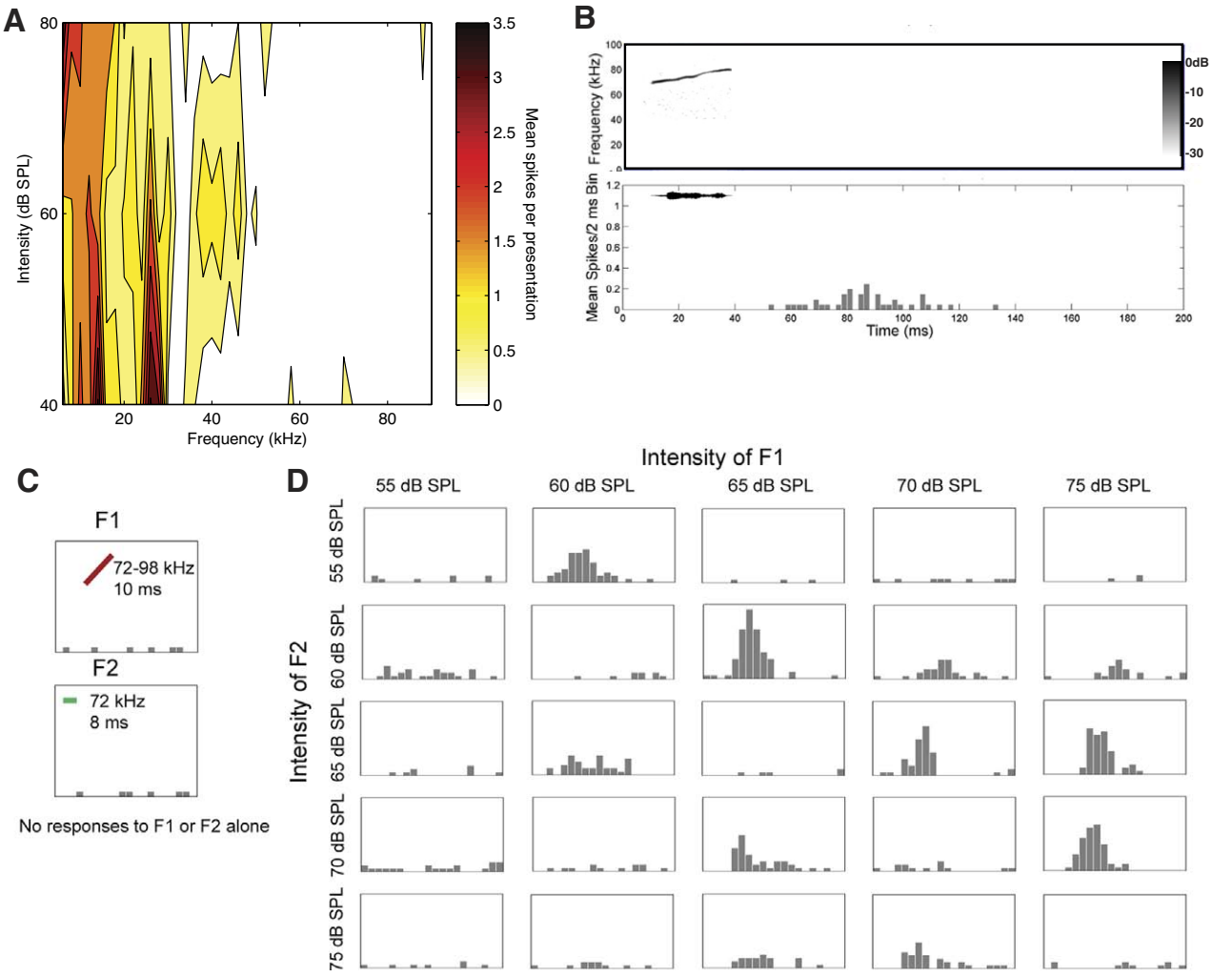


**Fig. 9.** Vocalization synthesized by combining a pure tone followed by a tone sweep. (A) Frequency tuning curve of a neuron in the low-broad tuning class. (B) Response of the neuron to a vocalization with energy only at frequencies higher than its frequency tuning curve. (C) The vocalization was synthesized by combining an 8 ms tone (F2) followed by a 10 ms frequency sweep (F1). There was no response to the individual elements of the synthesized stimulus. (D) The histograms show the spike responses to the combined tone-sweep with the elements presented at different intensities. The response was maximal when the intensity of F1 was larger than F2 by 5 dB SPL, as in the natural vocalization. The selectivity to the relative intensities of elements suggests that the neuron codes for the vocalization in an intensity independent manner.

tent of the vocalizations. We also tested each neuron for facilitatory interactions and found that facilitation did not explain the responses to the UHFVs in any of the neurons. Our findings suggest that the IC in mouse has evolved to detect behaviorally relevant vocalizations without devoting much space to the representation of frequencies contained in the majority of vocalizations.

Besides being able to detect ultrasonic vocalizations, we also found in this study that neurons in mouse IC are able to discriminate among different conspecific vocalizations that have similar spectrotemporal features. The level of selectivity varied between individual neurons with some neurons only responding to one or two vocalizations and other neurons responding to many or all. The mean SI value of 0.32 indicates that, on average, the neurons we recorded from responded to 11 of the 16 vocalizations. However, some neurons did show high levels of selectivity by responding to only one to three neurons.

The SI value is based solely on spike rate and does not provide any information on spike timing differences that may be important for discrimination among different conspecific vocalizations. In both auditory thalamus and auditory cortex, temporal discharge patterns provide reliable discriminations between natural and time-reversed vocalizations (Huetz et al., 2009; Schnupp et al., 2006). Consequently, to further our understanding of processing of vocalizations in IC, future studies should examine both spike rate and spike timing codes for discrimination of vocalizations.

Neural selectivity among different vocalizations has previously only been reported in the IC of bats (Klug et al., 2002; Portfors, 2004; Holmstrom et al., 2007; Andoni et al., 2007; Xie et al., 2005). Our findings of selectivity in mouse IC are significant because they show that bats are not the only species with specializations for encoding complex sounds at the level of the IC. It has been suggested that bats show more pronounced response specializations at

subcortical and primary cortical levels compared to monkeys because primates have more auditory areas beyond primary auditory cortex than non-primates (Kanwal and Rauschecker, 2007). Consequently, encoding of complex sounds such as vocalizations is relegated to higher cortical areas in primates and subcortical and cortical areas in non-primates (Kanwal and Rauschecker, 2007). Our findings support this idea in that response specializations for vocalizations appear in the IC of mice.

### Coding strategies

The finding that some neurons in the IC are selective to specific vocalizations reveals an important aspect of information coding in the midbrain. Complex sounds, and in particular behaviorally relevant sounds, are not coded solely by neurons responding to simple frequency characteristics found in the signals. However, it is important to note that these neurons are not so selective that they respond only to a single vocalization, but rather most respond to several vocalizations. A closer inspection of how the responses are distributed over the population of IC neurons reveals a general coding principle. Each vocalization elicits a response from a different set of neurons in the IC, and each neuron responds with a unique temporal pattern to each vocalization. The population code leads to a broad array of spatial–temporal patterns of activity in the IC to natural stimuli, a pattern that would be unique for each vocalization.

Although a rich pattern of neural responses that encode a sound is not surprising, the surprising discovery here is that the pattern of activity for vocalizations involves many more neurons than one would expect from the frequency tuning characteristics of neurons found in the IC using pure tones as stimuli. The encoding of natural vocalizations is over-represented in the IC, leading to a more refined encoding of subtle variations that are present in behaviorally relevant stimuli.

### Potential neural mechanisms underlying selectivity to vocalizations in IC

There are a number of potential mechanisms that may underlie neural selectivity to vocalizations in the IC. In some cases the selectivity of IC neurons to particular vocalizations can be fully or partially explained by inhibition surrounding the excitatory tuning curve (Klug et al., 2002; Xie et al., 2005), inhibition far from the excitatory tuning curve (Holmstrom et al., 2007) or nonlinear facilitation between multiple harmonics in the vocalization (Portfors, 2004). Although these mechanisms have all been described in bats, there is no reason to expect they are not also important for encoding vocalizations in other species. The IC of mice exhibits lateral inhibition (Egorova et al., 2001; Portfors and Felix, 2005) as well as combination-sensitive inhibition and facilitation (Portfors and Felix, 2005). Although we did not find that combination sensitivity was responsible for the responses to vocalizations obtained in the current study, the reason may be in our methodology. The role that combination sensitivity may play in creating selective responses in the IC of mice to vocalizations may be different than in bats because the acoustic characteristics of bat social vocalizations are quite different than mouse vocalizations. While both animals emit a rich repertoire of social vocalizations, bat vocalizations tend to have richer harmonic structure than mouse vocalizations (Kanwal and Rauschecker, 2007; Holy and Guo, 2005; Portfors, 2007). The harmonic structure means that facilitatory interactions often occur with two overlapping signals (Portfors, 2004). However, considering that many mouse vocalizations consist of frequency jumps, where frequencies can change 15–30 kHz in a few milliseconds (Holy and Guo, 2005; Portfors, 2007; Wang et al., 2008), neurons that exhibit both frequency and temporal combination sensitivity would be an efficient mechanism for creating selectivity to particular vocalizations. In the current study we only tested for combination-sensitive facilitation with overlapping tones. Thus, the extent that temporal combination-sensitive responses in the IC of mice are important for neural responses to vocalizations remains to be seen.

The above mechanisms however, all rely on there being some match between the frequency tuning curve of the neuron and the spectral content of the response-eliciting vocalization. Clearly this is not the case in many IC neurons in mouse. One possible means for neurons with low frequency tuning curves to respond to complex signals with much higher frequencies is via nonlinear distortion products generated in the cochlea. The generation of difference tones is a well-known phenomenon resulting from nonlinear distortion in the cochlea. Psychoacoustic studies have shown the perceptibility of difference tones by human observers (Plomp, 1965). Moreover, auditory nerve fibers respond to 2F2–F1 distortion products even when the two primary tones (F1 and F2) fail to generate a response in the nerve (Goldstein and Kiang, 1968). Recent evidence shows that IC neurons respond to cochlear distortions (Abel and Kossl, 2009; McAlpine, 2004). In the current study, we found that IC neurons respond to combinations of ultrasonic frequencies; frequencies contained in many behaviorally relevant vocalizations. Thus, the IC may take advantage of these cochlear distortions to broaden the representation of ultrasonic vocalizations rather than eliminating the distortions caused by transduction of auditory signals.

One may have expected that the auditory system has adapted to eliminate transduction distortions, such as intermodulation distortions, to encode an accurate image of the physical environment. However, there may be advantages in utilizing the effects of distortions to yield a broad band representation of natural sounds for more precise classification. The distortions will generate activity in neurons that would otherwise not be sensitive to the frequency content of the complex sound. Thus, a population code involving many more neurons than would be possible without the distortions may be generated in IC for encoding complex sounds. Moreover, our finding that distortion products are eliminated in some IC responses suggests that additional sculpting of responses occurs (likely through inhibition) such that the result is a rich population

coding of complex sounds in the IC, where each neuron has a unique response to each vocalization.

While we did not specifically demonstrate that cochlear distortions are utilized in the encoding of vocalizations in mouse IC, we did show that IC neurons respond to complex synthesized signals that generate cochlear distortions. In addition, the frequencies that generate distortions are not required to be simultaneous to generate responses in low-frequency-tuned neurons (Fig. 9). The likely explanation of these results is that responses to different frequencies resonate in the cochlea for several milliseconds to influence later frequencies rendering the combination detectable to neurons that would otherwise not respond to the individual tones. This responsiveness to non-simultaneous combinations of frequencies leads to a considerable expansion of the number of neurons in the IC that respond to behaviorally relevant vocalizations.

The findings of this study demonstrate that neurons in IC are heterogeneous in their responses to vocalizations. Some neurons respond to all vocalizations that have energy within the neuron's frequency tuning curve, some neurons are selective to particular vocalizations due to mechanisms such as inhibition and combination sensitivity, some may use cochlear distortions, and others may be responding to other specific spectral and/or temporal acoustic features within vocalizations. All of these possibilities create difficulties in teasing out the mechanisms underlying neural encoding of vocalizations. One means of further testing what acoustic features of vocalizations are responsible for driving neural responses in IC is to synthesize the vocalizations and then systematically manipulate individual spectral and temporal features to create variants of the vocalizations. Neural responses to the specific variants could then be compared to responses to the original signals to reveal how neurons in IC encode vocalizations. These types of results would further our understanding of how behaviorally relevant sounds are encoded by the auditory system.

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