

## **Binaural Unmasking of Frequency-Following Responses in Rat Amygdala**

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**Abstract**

Survival in natural environments for small animals such as rats often depends upon precise neural coding of life-threatening acoustic signals, and binaural unmasking of species-specific pain calls is especially critical. This study investigated how species-specific tail-pain chatter is represented in the rat amygdala, which receives afferents from both auditory thalamus and auditory association cortex, and whether the amygdaloid representation of the chatter can be binaurally unmasked. The results show that chatter with a fundamental frequency (F0) of 2.1 kHz was able to elicit salient phase-locked frequency-following responses (FFRs) in the lateral amygdala nucleus in anesthetized rats. FFRs to the F0 of binaurally presented chatter were sensitive to the interaural time difference (ITD), with the preference of ipsilateral-ear leading, as well as showing features of binaural inhibition. When interaurally correlated masking noises were added and ipsilateral chatter led contralateral chatter, introducing an ITD disparity between the chatter and masker significantly enhanced (unmasked) the FFRs. This binaural unmasking was further enhanced by chemically blocking excitatory glutamate receptors in the auditory association cortex. When the chatter was replaced by a harmonic tone complex with an F0 of 0.7 kHz, both the binaural-inhibition feature and the binaural unmasking were preserved only for the harmonic of 2.1 kHz but not the tone F0. These results suggest that both frequency-dependent ascending binaural modulations and cortical descending modulations of the precise auditory coding of the chatter in the amygdala are critical for processing life-threatening acoustic signals in noisy, and even reverberant environments.

**Keywords:** Amygdala; Auditory phase locking; Interaural time difference; Pain call; Precedence effect.

## INTRODUCTION

Perception of threatening sounds, such as predators' calls or species-specific pain calls, is vital for survival (Dennis and Melzack 1983; Hendrie et al. 1998). When such sounds occur in noisy environments, precise neural coding of these signals is even more critical. One audible and vowel-like component of the rats' vocal response to tail pain has been called "chatter" and is characterized by a fundamental frequency (F0) plus several harmonics (Jourdan et al. 1995). The latency of chatter to a pain stimulus is stable even when the pain stimulus is presented randomly relative to the respiratory rhythm of the rat (Jourdan et al. 1995). Thus chatter is not simply an artifact of quick breathing in response to pain. And since chatter intensity can be reduced by systemic administration of morphine (1-3 mg/kg), it has been suggested that chatter reflects an affective state of the rat (Jourdan et al. 1998). However, very few studies about either neural or behavioral responses to chatter have been found in the literature. Frequency-following responses (FFRs) are sustained potentials based on precisely phase-locked neural activities elicited by low-to-medium frequency periodical sound waveforms (Marsh et al. 1970; Smith et al. 1975). It is interesting and important to know whether FFRs to tail-pain chatter can be recorded in brain structures that process life-threatening signals.

The amygdala is importantly associated with emotion-relevant sound detection (Sander and Scheich 2001; Fecteau et al. 2007; Kuraoka and Nakamura 2007), auditory emotional learning (Davis 1994; LeDoux 2000), and fear-conditioned modulation of auditory coding (Quirk et al. 1997; Maren et al. 2001). For example, in humans the amygdala is involved in the perception of both positive and negative nonlinguistic emotional vocalizations (Fecteau et al. 2007). In rats, the lateral nucleus of the amygdala (LA) receives afferents from both

the medial division of the medial geniculate body (mMGB) of the auditory thalamus and the auditory association cortex (AAC) (LeDoux et al. 1990, Romanski and LeDoux 1993), and the majority of acoustically responsive LA cells respond to white noise in a sustained manner (Bordi et al. 1993). Calling responses to pain also enhance activity of the amygdala and elicit defense behaviors (Beckett et al. 1997). Thus the LA may be a critical structure processing tail-pain chatter. However, studies of rats auditory responses to chatter have not been found in the literature.

Due to the presence of extraneous noises in natural environments, pain-call signals in the amygdala must be unmasked to retain the most salient features. It has been well known that binaural hearing improves signal detection against background noise (Hirsh 1948), especially when the binaural configurations are different between signal and noise. FFRs recorded in the human brainstem are resistant to noise masking (Russo et al. 2004), and can be unmasked by binaural processing (Wilson and Krishnan 2005). Moreover, rats' tail-pain chatters are vowel-like with fundamental frequencies just above 2.0 kHz (Jourdan et al. 1995), which is within the frequency range for eliciting FFRs. Interestingly, in guinea pig mMGB, a substantial number of cells have phase-locked responses to tones with an upper-limiting frequency at 1,100 Hz and latencies between 7.5 and 11 ms (Wallace et al. 2007). Since the LA receives auditory projections directly from the mMGB, it is very possible that phase-locked responses also occur in the LA.

In this study we used a segment of a rat's tail-pain chatter or a tone complex as the target signals to investigate (1) whether the chatter or the tone complex can elicit FFRs in rat LA, (2) whether FFRs recorded in the LA are affected by binaural processing, and even unmasked by binaural processing, and (3) whether FFRs and/or binaural unmasking of

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FFRs recorded in the LA are cortically regulated.

## **MATERIALS AND METHODS**

### ***Animal preparation***

Fifty-eight young-adult male Sprague-Dawley rats (age 10-12 weeks, weight 300-350 grams) were used and treated in accordance with (1) the Guidelines of the Beijing Laboratory Animal Center, (2) the Policies on the Use of Animals and Humans in Neuroscience Research approved by the Society for Neuroscience (2006), and (3) the Guiding Principles in the Care and Use of Animals provided by the American Physiology Society.

Rats were anesthetized with 10% chloral hydrate (400 mg/kg, intraperitoneal) and the state of anesthesia was maintained throughout the experiment by supplemental injection of the same anesthetic. Stainless steel recording electrodes (10-20 k $\Omega$ ) insulated by a silicon tube (0.3 mm in diameter) except at the 0.25 mm diameter tip (Zheng et al., 2008) were aimed at unilateral LA in the 58 rats and injection guild cannulae (C317G guide Cannula, Plastics One inc, VA, USA) were aimed at bilateral temporal cortex area TE3 in 46 rats based on the stereotaxic coordinates of Paxinos and Watson (1997). Referenced to Bregma, LA coordinates were: AP, -2.8 to -3.1 mm; ML,  $\pm$ 5.2 to 5.4 mm; DV, -7.6 to -8.1 mm; TE3 coordinates were: AP, -5.8 mm; ML,  $\pm$ 6.5 mm; DV, -5.5 mm. Note that area TE3 in rats is the major AAC that projects to the LA (Romanski and LeDoux 1993; Shi and Cassell 1997).

### ***Acoustic stimulation and electrophysiological recording***

Two kinds of signals were used in this study:

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(1) A train of tail-pain-chatter bursts was recorded from one rat in response to tail-clamping pain in a soundproof chamber and digitized at 44.1-kHz sampling rate and 16-bit resolution. A 150-ms stimulus section without any amplitude- or frequency-modulation was isolated within one chatter burst and tapered with 5-ms linear onset/offset ramps. The spectrum of the chatter shows a fundamental frequency (F0) at 2.1 kHz, and two harmonics at 4.2 (h2) and 6.3 kHz (h3) (Figure 1A).

----- Insert Figure 1 about here -----

(2) A tone complex with a duration of 150 ms was digitally generated by the Cooledit audio editing software at 44.1-kHz sampling rate and 16-bit resolution, and tapered with 5-ms linear onset/offset ramps. The spectrum of the tone complex shows a fundamental frequency (F0) at 0.7 kHz and two harmonics at 2.1 (h3) and 3.5 kHz (h5) (Figure 1B). Note that the h3 component of the tone complex shared the same frequency with the F0 component of the chatter.

The masker was a broadband noise (0-10 kHz) with a duration of 750-ms (including 5-ms linear onset/offset ramps), beginning 500 ms before the onset of the chatter or the tone complex.

All sound waves were processed by the TDT System II (Tucker-Davis Technologies, FL, USA), and presented through two ED1 earphones. A 12-cm TDT sound-delivery rubber tube was connected to each ED1 earphone and inserted into the left and right ear canal. Signals in quiet and in noise at the tube ends were calibrated using a Larson Davis Audiometer Calibration and Electroacoustic Testing System (AUDit™ and System 824,

Larson Davis, USA). The sound-pressure level (SPL) of the signal (chatter or tone complex) in quiet was fixed at 59 dB when each earphone played alone. Under conditions with masking noise presentations, the signal intensity was held constant at this level while that of the masking noise was adjusted to produce two signal-to-noise ratios (SNRs): -4 and 4 dB.

Neural potentials to acoustic stimulation were recorded in the LA via the stainless steel

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(2) Locke's/0.1-ms ( $n = 9$ ), (3) KYNA/1-ms ( $n = 14$ ), and (4) Locke's/1-ms ( $n = 9$ ). There was also a masker-control group (see below for details) which did not receive any injection ( $n = 12$ ).

Two blocks of recordings were conducted before injection with the chatter being the signal in one block and the tone complex being the signal in the other block. One additional block of recording was conducted after injection only with the chatter as the signal.

In each block before injection, rats were adapted to the signal for 10 min, then the following stimuli were presented: (1) monaural signal (ipsilateral, I; contralateral, C) in quiet, (2) binaural signal in quiet with the following ITDs: -0.1 or -1 ms (ipsilateral signal leading, I/C), 0 ms (bilaterally simultaneous, ST), and +0.1 or +1 ms (contralateral signal leading, C/I), and (3) binaural signal in masking noise (the four groups with injection were presented with both the signal (chatter or tone complex) and interaurally correlated noise, while the masker-control group was presented with both the chatter and interaurally uncorrelated noise).

Under conditions with co-presentation of masking noise, when the ITD for signal was -0.1 or +0.1 ms and the ITD for interaurally correlated noise was -0.1, 0, or +0.1 ms, there were three *absolute* ITD disparities between the signal and noise ( $|ITD_{S+N}|$ ): 0 (no ITD disparity), 0.1 (smaller ITD disparity) and 0.2 ms (larger ITD disparity). Similarly, when the ITD for signal was -1 or +1 ms, and the ITD for masking noise was -1, 0, or +1 ms, three types of  $|ITD_{S+N}|$  were: 0, 1, and 2 ms. Note that the ITD value of 0.1 ms was shorter than the maximum ITD due to the head size of rats (0.13 - 0.16 ms, Koka et al., 2008), and the ITD value of 1 ms was within the sound delay range for inducing fused sound images in behaving rats (Kelly 1974; Hoeffding and Harrison 1979). Thus we assume that for awake



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rats when  $|\text{ITD}_{\text{S+N}}|$  is zero, no separation is perceived between signal image and noise image; when  $|\text{ITD}_{\text{S+N}}|$  is 0.1 or 1 ms, the signal is perceived as being at one ear and the noise is at the center of the head (smaller perceived signal/noise separation); when the  $|\text{ITD}_{\text{S+N}}|$  is 0.2 or 2 ms, the signal is perceived at one ear and the noise is perceived at the other ear (larger perceived signal/noise separation).

The inter-stimulus interval (ISI) was 800 ms for signals presented in quiet and 1000 ms for signals presented in noise. FFRs in quiet were recorded for a duration of 200 ms beginning at signal onset, while FFRs in noise were recorded for a duration of 800 ms beginning at noise onset. Recordings were carried out before and after microinjection of KYNA or Locke's solution in area TE3 for each of the four groups with injection.

### *Data analyses*

For acoustically evoked potentials recorded in quiet, a 1000-Hz low-pass filter was used to smooth the potential waveform. The latency of monaural chatter- or tone-elicited field potentials in quiet was determined by measuring the time interval between the sound onset and the first positive peak (P1, Figure 3A) of the response waveform. The latency of the primary negative peak (PN, Figure 3A) was also determined. Fast-Fourier transform (FFT) was performed for each unfiltered waveform in quiet (Figure 3E-H). The spectral peak amplitude of a 100-Hz-wide frequency band centered at 2.1 and 4.2 kHz in response to chatter were determined as the FFR F0 and h2 amplitudes, respectively. The spectral peak amplitude of a 100-Hz-wide frequency band centered at 0.7 and 2.1 kHz in response to the tone complex were also determined as the FFR F0 and h3 amplitudes, respectively.

For acoustically evoked potentials recorded in noise, FFT was performed during a

period from the signal onset to 15 ms after the signal offset. When chatter was the signal, the spectral peak amplitude of a 100-Hz-wide band centered at 2.1 kHz was determined and labeled as the FFR F0 amplitude of the signal in noise (AMPs+n), and the mean spectral amplitude of two 200-Hz-wide sidebands centered at 1.95 kHz and 2.25 kHz was defined as the amplitude of noise (AMPn). The response signal-to-noise ratio (rSNR) was defined as AMPs+n /AMPn. Computations were also done separately for the 0.7-kHz (F0) FFRs and the 2.1-kHz (h3) FFRs to the tone complex.

The unmasking index, UI, which was used to evaluate the effect of ITD disparity between signal and masker on FFR, was then calculated as:

$$UI (\%) = \frac{\frac{rSNR_{-4/N} - rSNR_{-4/0}}{rSNR_{-4/0}} + \frac{rSNR_{4/N} - rSNR_{4/0}}{rSNR_{4/0}}}{2} \times 100 \%$$

Where, “-4” and “4” represent the stimulus sSNR of -4 and +4 dB, respectively. “0” represents the zero ITD disparity ( $|ITD_{S+N}| = 0$ ), and “N” represents a particular ITD disparity, (0.1, 0.2, 1, or 2 ms). “rSNR<sub>-4/0</sub>”, for example, represents the response SNR when the stimulus SNR was -4 dB and the zero disparity was introduced.

### *Statistical analyses*

Analyses of variance (ANOVAs) and Bonferroni post hoc tests were performed by using SPSS 13.0 software (for details see Results). The null-hypothesis rejection level was set at 0.05.

### ***Histology***

When all recordings were finished, rats were killed with an overdose of chloral hydrate. Lesion marks were made via the recording electrodes by an anodal DC current (500  $\mu$ A for 10 s). The brains were stored in 10% formalin with 30% sucrose, and then sectioned at 40  $\mu$ m in the frontal plane in a cryostat (-20°C). Sections were examined to determine locations of recording electrodes and injection cannulae.

### **RESULTS**

According to histological examination (Fi

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positive peak potential was 8.03 ms (SD = 0.68 ms) and the mean latency for the primary negative peak was 10.83 ms (SD = 0.97 ms). For the onset response to the tone complex, the mean latencies of these two peak potentials were 7.67 ms (SD = 0.42 ms) and 10.92 ms (SD = 0.76 ms), respectively.

----- Insert Figure 3 about here -----

### ***Monaural and binaural FFRs when no noise masker was presented***

FFRs to ipsilateral, contralateral, or bilateral stimulation were first analyzed for conditions without the presentation of noise masker. When the noise masker was not presented, fast-Fourier spectral analyses of field-potential waveforms to the chatter presented at either the contralateral or ipsilateral ear (Figure 3A, B) clearly revealed the F0 component in all of the 51 rats, but the h2 component was found only in 22 (43%) rats and the h3 component was not detected in any recording sites (Figure 3E, F). Fast-Fourier spectral analyses of field-potential waveforms to the tone complex presented at either the contralateral or ipsilateral ear (Figure 3C, D) revealed both the F0 and h3 components in all 51 rats, and the h5 component in 31 (61%) rats. Since the chatter-h3 and tone-h5 components could not be reliably elicited across animal subjects, this study only focused the analyses on the tone-F0, tone-h3 and, chatter-F0 components.

FFRs to the ipsilateral chatter F0 or to the ipsilateral tone h3 were larger than those to the contralateral chatter F0 or to the contralateral tone h3 (see Figure 3, and Stimulus Conditions I and C in Figure 4).

Binaural interaction was examined by presenting the signal (chatter or tone complex) at

the two ears and manipulating the ITD. Figure 4 shows mean normalized spectral amplitudes of FFRs to the tone F0 (Figure 4A, D), tone h3 (Figure 4B, E), and chatter F0 (Figure 4C, F) under various monaural/binaural stimulation conditions across rats from different groups before the injection manipulation was conducted. Presenting the signal only at the contralateral ear (Condition C) served as the baseline condition (e.g., amplitude = 1) for normalization in each panel.

----- Insert Figure 4 about here -----

Three (frequency component: tone F0, tone h3, chatter F0) five (stimulation condition: I/C, ST, C/I, I, C) within-subject ANOVAs were conducted for rats with the ITD of 0.1 ms and 1 ms separately. Results show that the interaction between frequency and stimulation condition was significant for 0.1-ms ITD [ $F(8, 12) = 29.940, P < 0.0001$ ] and 1-ms ITD [ $F(8, 12) = 6.595, P = 0.002$ ].

For chatter-F0 amplitudes in rats with the ITD of 0.1 ms (Figure 4C) and those with the ITD of 1 ms (Figure 4F), one-way ANOVAs indicate significantly different F0 amplitudes across various monaural/binaural conditions [0.1-ms ITD:  $F(4, 95) = 19.505, P < 0.0001$ ; 1-ms ITD:  $F(4, 95) = 15.573, P < 0.0001$ ]. Post hoc tests show that (1) F0 amplitude under Condition I (ipsilateral only) was significantly larger than that under Condition C (contralateral only) ( $P < 0.0001$  for each of the two ITDs); (2) F0 amplitude under Condition I/C (ipsilateral chatter leading contralateral) was significantly larger than that under Condition ST (simultaneous between the two ears) as well as that under Condition C/I (contralateral chatter leading ipsilateral) (all  $P < 0.01$ ); (3) F0 amplitude under

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Condition ST was not different from that under Condition C/I ( $P > 0.05$  for each of the two ITDs). These results confirm a remarkable ipsilateral-input dominance in eliciting FFRs to the chatter F0. Moreover, compared to the F0 amplitude under Condition I, adding the contralateral chatter either 1 or 0 ms before the ipsilateral chatter significantly decreased the F0 amplitude (all  $P < 0.0001$ ), showing an ITD-dependent binaural inhibition. However adding contralateral chatter either 0.1 or 1 ms after ipsilateral chatter did not significantly change F0 amplitude ( $P > 0.05$  for each group).

Similarly, for tone-h3 amplitudes in rats with the ITD of 0.1 ms (Figure 4B) and rats with the ITD of 1 ms (Figure 4E), one-way ANOVAs also indicate remarkably different h3 amplitudes across monaural/binaural conditions [0.1-ms ITD:  $F(4, 95) = 20.285$ ,  $P < 0.0001$ ; 1-ms ITD:  $F(4, 95) = 9.396$ ,  $P < 0.0001$ ]. Post hoc tests show that (1) the h3 amplitude under Condition I was significantly larger than that under Condition C ( $P < 0.0001$  for each of the two ITDs); (2) the h3 amplitude under Condition I/C was significantly larger than that under Condition ST and that under Condition C/I (all  $P < 0.05$ ); (3) the h3 amplitude under Condition ST did not significantly differ from that under Condition C/I ( $P > 0.05$  for each of the two ITDs). These results also confirm an ipsilateral-input dominance in tone-h3-elicited FFRs. Moreover, compared to the h3 amplitude under Condition I, adding the contralateral tone either 1 or 0 ms before ipsilateral tone significantly decreased the h3 amplitude (all  $P < 0.0001$ ), while adding contralateral tone either 0.1 or 1 ms after ipsilateral tone did not change the h3 amplitude ( $P > 0.05$  for each group). Thus the tone-h3 amplitude for each of the two ITDs also shows an ITD-dependent binaural inhibition.

For tone-F0 amplitudes in rats with the ITD of 0.1 ms (Figure 4A) and those with the ITD of 1 ms (Figure 4D), one-way ANOVAs show significantly different F0 amplitudes

across monaural/binaural conditions [0.1-ms ITD:  $F(4, 95) = 6.808, P < 0.0001$ ; 1-ms ITD:  $F(4, 95) = 4.165, P < 0.01$ ]. Post hoc tests for rats with 0.1-ms ITD show that F0 amplitudes under each of the three binaural conditions were significantly higher than those under each of the two monaural conditions (all  $P < 0.05$ ). Post hoc tests for rats with 1-ms ITD show that F0 amplitudes under Condition I/C were significantly larger than those under Condition I ( $P < 0.05$ ) and Condition C ( $P < 0.01$ ), indicating an ITD-dependent binaural summation effect but not an ipsilaterally dominant effect. The differences between tone-h3 and tone-F0 amplitudes suggest differentiated binaural modulations of these two frequency components.

#### ***Effects of blocking AAC on FFRs to chatter presented in quiet***

Figure 5 shows normalized chatter-F0 amplitudes under various monaural/binaural stimulation conditions before (diagonal bars) and after (empty bars) either KYNA (Figure 5A, C) or Locke's solution (Figure 5B, D) was bilaterally injected into temporal cortex area TE3, when the noise masker was not presented. The chatter-F0 amplitude under Condition C before injection served as the baseline condition for normalization.

----- Insert Figure 5 about here -----

For each of the following three animal groups: KYNA/0.1-ms (Figure 5A), Locke's/0.1-ms (Figure 5B), and Locke's/1-ms (Figure 5D), two (injection: pre, post) by five (stimulation condition) repeated-measures ANOVAs show that neither the main effect of injection nor the interaction between injection and conditions was significant (all  $p >$

0.05), but the main effect of stimulus condition was significant [KYNA/0.1-ms:  $F(4, 8) = 97.621$ ; Locke's/0.1-ms:  $F(4, 4) = 76.316$ ; Locke's/1-ms:  $F(4, 4) = 85.834$ ; all  $P < 0.0001$ ]. Thus for each of these three groups, the injection manipulation did not significantly change the FFR profiles. For the KYNA/1-ms group (Figure 5C), a repeated-measures ANOVA shows that the main effect of injection was significant [ $F(1, 11) = 6.671$ ,  $P = 0.025$ ], the main effect of stimulus condition was significant [ $F(4, 8) = 48.391$ ,  $P < 0.0001$ ], but the interaction between injection and stimulus condition was not significant ( $P > 0.05$ ). Thus for this group, bilateral injection of KYNA into area TE3 decreased the F0 amplitude generally.

These results indicate that when the noise masker was not presented, neither the ipsilateral-input dominant feature nor the binaural interaction was changed by bilateral injection of either KYNA or Locke's solution into area TE3.

### ***Binaural unmasking of FFRs to chatter and FFRs to tone complex***

As mentioned above, FFRs recorded in the human brainstem can be unmasked by binaural processing (Wilson and Krishnan 2005). The present study also investigated whether FFRs in rat LA can be binaurally unmasked. Since FFRs recorded in the LA were ipsilateral-input dominant, results of binaural unmasking of FFRs are reported here only for conditions when signals at the ipsilateral ear led the contralateral ear by either 0.1- or 1-ms.

Figure 6 shows the mean unmasking indices (UIs) induced by a non-zero ITD disparity between the signal and masker across different groups before the injection manipulation was conducted for the tone F0 (Figure 6A, D), tone h3 (Figure 6B, E), and chatter F0



(Figure 6C, F). Clearly, distinct and positive UIs occurred for both the chatter-F0 and tone-h3 components, but not the tone-F0 component.

One-way ANOVAs show that relative to the zero ITD disparity ( $|ITD_{S+N}| = 0$ ), introducing a non-zero ITD disparity ( $|ITD_{S+N}| = 0.1, 0.2, 1, \text{ or } 2 \text{ ms}$ ) between the signal and masker markedly enhanced the chatter-F0 and tone-h3 amplitudes, but not the tone-F0 amplitude [chatter-F0/0.1-ms:  $F(2, 57) = 25.281, P < 0.0001$ ; chatter-F0/1-ms:  $F(2, 57) = 33.715, P < 0.0001$ ; tone-h3/0.1-ms:  $F(2, 57) = 12.221, P < 0.0001$ ; tone-h3/1-ms:  $F(2, 57) = 16.314, P < 0.0001$ ; tone-F0/0.1-ms:  $F(2, 57) = 0.218, P = 0.447$ ; tone-F0/1-ms:  $F(2, 57) = 0.119, P = 0.888$ ]. Post hoc tests show that the tone-h3 amplitude was significantly enhanced when  $|ITD_{S+N}|$  was 0.1, 1, or 2 ms, while the chatter-F0 amplitude was significantly enhanced under each of the non-zero  $|ITD_{S+N}|$  conditions (all  $P < 0.01$ ).

To compare differences in FFR enhancement between chatter F0 and tone h3, and differences between smaller  $|ITD_{S+N}|$  and larger  $|ITD_{S+N}|$  (i.e., 0.1 ms vs. 0.2 ms; 1 ms vs. 2 ms), two two-by-two ANOVAs were conducted:

(1) For rats with the signal ITD of 0.1 ms, a two (signal type: tone-h3, chatter-F0) by two (non-zero  $|ITD_{S+N}|$ ) ANOVA shows that the main effect of signal type was significant [ $F(1, 76) = 17.102, P < 0.0001$ ], the main effect of  $|ITD_{S+N}|$  was significant [ $F(1, 76) = 12.449, P = 0.001$ ], but the interaction between signal type and  $|ITD_{S+N}|$  was not significant [ $F(1, 76) = 0.002, P = 0.967$ ]. The results indicate that introducing a non-zero ITD disparity caused a significantly larger enhancement of the chatter-F0 amplitude than that of the tone-h3 amplitude, and introducing the  $|ITD_{S+N}|$  of 0.1 ms caused a significantly larger enhancement in chatter-F0 or tone-h3 amplitude than introducing the  $|ITD_{S+N}|$  of 0.2 ms.

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(2) For rats with the signal ITD of 1 ms, a two (signal type: tone-h3, chatter-F0) by two (non-zero  $|ITD_{S+N}|$ ) ANOVA shows that the main effect of  $|ITD_{S+N}|$  was significant [ $F(1, 76) = 6.051, P = 0.016$ ], but the main effect of signal type was not significant [ $F(1, 76) = 1.463, P = 0.230$ ], and the interaction between signal type and  $|ITD_{S+N}|$  was not significant [ $F(1, 76) = 0.141, P = 0.708$ ]. The results indicate that introducing a non-zero ITD disparity caused an equal enhancement in the chatter-F0 amplitude and the tone-h3 amplitude, and introducing the  $|ITD_{S+N}|$  of 1 ms caused a significantly larger enhancement in chatter-F0 or tone-h3 amplitude than introducing the  $|ITD_{S+N}|$  of 2 ms.

----- Insert Figure 6 about here -----

The role of binaural interaction in improving FFRs was further supported by the results from the masker-control group (Figure 7) in which masking noises at the two ears were uncorrelated (independent). For this animal group, the UI under either 1-ms- $|ITD_{S+N}|$  or 2-ms- $|ITD_{S+N}|$  condition was not significant [ $P > 0.05$ ], indicating that the binaural unmasking effect did not occur when noises at the two ears were not correlated.

----- Insert Figure 7 about here -----

### *Effects of blocking ACC on binaural unmasking of FFRs to chatter*

Whether binaural unmasking of FFRs to the chatter is cortically modulated was examined by bilateral injection of KYNA (Figure 8A, C) or Locke's solution (Figure 8B, D) into the cortical area TE3. As shown by Figure 8, injection of KYNA but not Locke's solution into the cortical area enhanced the UI.

For the KYNA/0.1-ms group (Figure 8A), a two (injection: pre, post)  $\times$  two (non-zero  $|ITD_{S+N}|$ ) repeated-measures ANOVA shows that the main effect of injection was significant [ $F(1, 11) = 7.910, P = 0.017$ ], the main effect of  $|ITD_{S+N}|$  was not significant [ $F(1, 11) = 1.074, P = 0.322$ ], but the interaction between injection and  $|ITD_{S+N}|$  was significant [ $F(1, 11) = 7.728, P = 0.018$ ]. Separate paired-sample t-tests show that KYNA injection had no significant effect on UI under the 0.1-ms- $|ITD_{S+N}|$  condition [ $t(11) = 0.790, P > 0.05$ ] but significantly increased UI under the 0.2-ms- $|ITD_{S+N}|$  condition [ $t(11) = 4.614, P = 0.001$ ].

For the KYNA/1-ms group (Figure 8C), a two (injection: pre, post)  $\times$  two (non-zero  $|ITD_{S+N}|$ ) repeated-measures ANOVA shows that the main effect of injection was significant [ $F(1, 11) = 23.141, P = 0.001$ ], the main effect of  $|ITD_{S+N}|$  condition was not significant [ $F(1, 11) = 1.710, P = 0.218$ ], and the interaction between injection and  $|ITD_{S+N}|$  was not significant [ $F(1, 11) = 0.480, P = 0.503$ ]. Separate paired-samples t-tests confirm that KYNA injection significantly enhanced UI under both the 0.1-ms- $|ITD_{S+N}|$  condition [ $t(11) = 2.773, P = 0.018$ ] and the 0.2-ms- $|ITD_{S+N}|$  condition [ $t(11) = 7.339, P < 0.001$ ].

For two Locke's-injection groups (Figure 8B, D), two (injection)  $\times$  two ( $|ITD_{S+N}|$ ) repeated-measures ANOVAs show that the main effect of injection, the main effect of

$|ITD_{S+N}|$ , and the effect of interaction between injection and  $|ITD_{S+N}|$  were not significant (all  $P > 0.05$ ).

----- Insert Figure 8 about here -----

## **DISCUSSION**

### ***Short onset-response latency***

In this study, both the chatter and the tone complex presented at either ear were able to evoke marked field potentials in the LA. However, the stimulus at the contralateral ear, but not at the ipsilateral ear, elicited marked onset responses. The latency of the first positive peak to acoustic stimuli at the contralateral ear was about 8 ms. This short onset-response latency suggests fast pathways connecting the cochlear nucleus to the mMGB and then to the LA. Indeed, it has been shown in rats that the dorsal cochlear nucleus, the small cell cap of the ventral cochlear nucleus, and the posterior ventral cochlear nucleus directly project to the mMGB bypassing the auditory midbrain (Malmierca et al. 2002). This fast neural connection would be critical for ensuring immediate LA processing of environmental acoustic signals.

### ***Frequency dependence and ipsilateral-input dominance of FFRs***

For the chatter stimulus, the F0 component (2.1 kHz) elicited FFRs in all recorded sites in the LA, but the h2 component (4.2 kHz) elicited FFRs only in 22 out of the 51 recorded sites, indicating that phase-locked neural responses in the LA reliably preserve the F0

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component as the most salient chatter feature. For the tone complex stimulus, although both the F0 component (0.7 kHz) and the h3 component (2.1 kHz) elicited FFRs in all the recorded sites and the h5 component (3.5 kHz) elicited FFRs in most of the recorded sites, the amplitude of the h3 component appears to be larger than that of the F0 component and that of the h5 component (Figure 3G, H), suggesting that phase-locking processing in the LA is more sensitive to the F0 frequency of species-specific tail-pain chatter.

The mMGB, which mainly receives its ascending inputs from the ipsilateral auditory midbrain (the inferior colliculus, IC) and contralateral cochlear nucleus (LeDoux et al. 1987, 1990; Malmierca et al. 2002), transfers auditory signals to the ipsilateral LA (LeDoux et al. 1990). In the rat's IC, the majority of neurons responding to sounds (including EE, EI, and EO neurons) are excited by stimulation of the contralateral ear, but only a small proportion of neurons, termed EE cells, have excitatory responses to stimulation of the ipsilateral ear (Flammino and Clopton 1975; Kelly et al. 1991; Li and Kelly 1992a; Sally and Kelly 1992; Silverman and Clopton 1977). Thus it would be predicted that FFRs recorded in the LA would be stronger when the contralateral ear was stimulated than when the ipsilateral ear was stimulated. Surprisingly, although the onset component of field potentials to contralateral stimulation was much larger than that to ipsilateral stimulation, for the F0 component in FFRs to chatter and the h3 component in FFRs to the tone complex, the signal presented at the ipsilateral ear elicited significantly larger spectral amplitudes than that at the contralateral ear (stimulus conditions I and C in Figure 4B, C, E, F), showing a marked ipsilateral-input dominance in FFRs. However, for the F0 component in FFRs to the tone complex, this ipsilateral dominance did not occur (Figure 4A, D). These interesting results suggest that inputs from the contralateral ears are used by the LA largely for fast

detecting the occurrence (onset) of an acoustic event and inputs from the ipsilateral ear are used largely for analyzing the acoustic details that code critical species-specific information.

What are the possible mechanisms underlying the ipsilateral dominance of FFRs in the LA? The LA on the left side of the brain, for example, mainly receives auditory inputs from the ipsilateral (left) mMGB (LeDoux et al. 1990), which in turn is mainly driven by outputs from the ipsilateral (left) IC. As mentioned above, the only type of IC neurons that can be excited by stimulation at the ipsilateral ear are EE neurons. Thus ipsilaterally-driven FFRs in the LA are based on outputs from EE neurons in the ipsilateral IC. On the other hand, all types of neurons in the ipsilateral (left) IC, including EE, EI, and EO neurons, are excited by sounds at the contralateral (right) ear. Thus contralaterally-driven FFRs in the LA are based on outputs from various types of neurons in the ipsilateral IC. In other words, LA FFRs driven by the ipsilateral ear have a lower degree of input convergence than contralaterally-driven LA FFRs. Since FFRs reflect the phase-locking synchronization of a local neuron population, it is suggested that because more neuron types are involved in providing information from the contralateral ear to the LA than from the ipsilateral ear, the phase-locking synchronization of neural populations firing to contralateral stimulation fades faster than that to ipsilateral stimulation during the course of signal transformation from the IC to LA, leading to the ipsilateral-input dominance in inducing FFRs in the LA. The lack of ipsilateral dominance of FFRs in the LA for the tone component of 0.7 kHz implies that FFRs below 1 kHz are mainly contributed by EE neurons with lower characteristic frequencies (see Kelly et al. 1991). Clearly, in future studies single-unit recordings from the LA are needed to examine whether action-potential spikes to ipsilateral stimulation and those to contralateral stimulation are different in vector strength value

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(which estimates the degree of phase locking) and/or in the variety of preferred firing phase.

### ***Binaural interactions in FFRs***

Although the chatter and the tone complex presented at either ear was able to elicit FFRs in the LA, overall binaural facilitation was observed only for the tone F0 component with the low frequency of 0.7 kHz (Figure 4A, D shows significant differences between binaural and monaural conditions, but not within binaural conditions), but not for the tone h3 or the chatter F0 components. Thus only the tone F0 component showed significantly larger FFR amplitudes in the binaural as compared with the monaural stimulus conditions. As discussed above, FFRs below 1 kHz may be mainly attributed to EE neurons with lower characteristic frequencies. Thus, the binaural facilitation of FFRs may reflect binaural summation of low-frequency EE neurons in the IC.

However, FFRs associated with the chatter F0 and tone h3 components, but not the tone F0 component, exhibited marked binaural inhibition under conditions when the contralateral stimulus was presented no later than the ipsilateral stimulus (stimulus conditions ST and C/I in Figure 4), because the spectral amplitude under the binaural stimulation conditions when the ipsilateral stimulus did not lead was lower than that under the ipsilateral leading condition (stimulus condition I/C). It should be noted that the binaural inhibition of FFRs in the LA may not reflect the binaural inhibitory EI responses in the IC, because binaural EI neurons in the IC are excited by stimulation of the contralateral ear and inhibited by stimulation of the ipsilateral ear (Kelly et al. 1991) and GABAergic axonal projections to the IC from the contralateral dorsal nucleus of the lateral lemniscus (DNLL) play a critical role in forming the EI response pattern in the IC (Li and Kelly

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1992b; Kelly and Li 1997; Zhang et al. 1998).

What are the possible mechanisms underlying the binaural inhibition in LA FFRs? As mentioned before, in the rat's IC almost all types of auditory neurons are excited by stimulation of the contralateral ear but only EE neurons are excited by stimulation of the ipsilateral ear, and the ipsilateral dominance of monaural LA FFRs reflects better phase-locking synchronization in the LA associated with ipsilaterally-driven EE neurons. Adding the stimulus at the contralateral ear may increase the diversity of auditory inputs to the LA and reduce the phase-locking synchronization of the LA neuron population, particularly when the contralateral stimulus leads the ipsilateral stimulus.

On the other hand, the ipsilaterally-induced neural excitation in the IC is modulated by both the contralateral IC via the commissure of inferior colliculus (which contain excitatory projecting axons between the two IC; Malmierca et al. 2003, 2005) and the contralateral DNLL via the commissure of Probst (which contains inhibitory projecting axons from the contralateral DNLL to the IC; Zhang et al. 1998). Our recent studies have shown that ipsilaterally-induced FFRs recorded in the IC are weakened by injection of KYNA into the contralateral IC but enhanced by injection of KYNA into the contralateral DNLL (Ping et al. 2008), indicating that (1) the contralateral IC facilitates ipsilaterally-driven IC FFRs via the excitatory axonal components in the commissure of inferior colliculus, and (2) the contralateral DNLL inhibits ipsilaterally-driven IC FFRs via the commissure of Probst. Thus it is speculated that when FFRs in the left LA, for example, are induced by a stimulus (e.g., the chatter) at the ipsilateral (left) ear, this stimulus also excites both the contralateral (right) IC and the contralateral (right) DNLL. If the same stimulus is also delivered to the contralateral ear, this contralateral stimulus inhibits the contralateral (right) IC because it



excites the ipsilateral (left) DNLL, leading to a reduction of inter-colliculus facilitation of ipsilaterally-driven IC FFRs.

Since binaural inhibition in rats is related to sound localization (e.g., Kelly et al. 1996), the results obtained in this study suggest that the neural mechanisms underlying FFRs recorded in the LA are also associated with processing certain spatial information. In other words, binaural inhibition of FFRs in the LA may be important for rats to encode both fine-structure and spatial features of pain calls and allocate spatial attention to the salient chatter. For example, when a rat faces two different chatters from different spatial locations, binaural inhibition that occurs in the LA may lead to a difference in the neural representation of the fundamental frequencies between the two sides of LA, allowing the rat to more effectively perceive the chatter based on a higher sound level and/or earlier arrival. Meck and MacDonal (2007) recently proposed that under stressful conditions the amygdala is crucial for disrupting simultaneous temporal processing of two or more signals in order to mediate fear-related selective attention to the most salient signal and ignore other signals that emerge simultaneously. Thus the binaural inhibition feature of FFRs in LA may also be associated with modulation of spatial attention to signals details.

### ***Binaural unmasking***

As mentioned in the Introduction, binaural hearing unmask signals against background noise when binaural configurations are different between signal and noise (Hirsh 1948). In humans, brainstem FFRs are resistant to noise masking (Russo et al. 2004) and unmasked by binaural processing (Wilson and Krishnan 2005). Results of the present study clearly indicate that when ipsilateral chatter leads contralateral chatter, FFRs in LA can be

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improved by introducing an ITD disparity between the chatter (or tone complex) and interaurally correlated noise. However, the binaural unmasking is frequency dependent, because in this study (Figure 6) it occurred only for the 2.1-kHz component (chatter F0 and tone h3) but not for the 0.7-kHz component (tone F0).

This binaural unmasking was further confirmed by the absence of an unmasking effect when the noises at the two ears were uncorrelated (Figure 7). Since binaural masking level difference effects have been well demonstrated in guinea pig's IC (e.g., Caird et al. 1991) and chinchilla's IC (e.g., Mandava et al. 1996), the unmasking of FFRs in the LA may reflect the binaural unmasking process in the IC. Interestingly, results of this study also show that under the ITD conditions of 0.1 or 1 ms, for both the chatter F0 and tone h3 components, introducing the smaller ITD disparity (0.1 or 1 ms) caused a significantly larger enhancement in the FFR amplitude than introducing the larger ITD disparity (0.2 or 2 ms).

In humans, when both an acoustic signal (i.e., speech) and a masker are presented by each of two spatially separated loudspeakers, perceived spatial separation between signal image and masker image, which is caused by perceptual fusion of correlated waveforms, significantly improves recognition of the signal (e.g. Freyman et al. 1999, Li et al. 2004; Wu et al. 2005). The binaural unmasking of FFRs in the LA as revealed by this study may be associated with perceptual fusion of correlated sounds (Kelly 1974; Hoeffding and Harrison 1979). Indeed, when the masking noises at the two ears were uncorrelated and not fused, which leads to no perceived separation between the signal and noise, the binaural unmasking effect disappeared.

### ***Specificity of FFRs to the chatter***

In this study, a tone complex, with an F0 of 0.7 kHz, h3 of 2.1 kHz, and h5 of 3.5 kHz, was used as a control stimulus for examining the specificity of FFRs to the chatter F0 component (2.1 kHz). When the noise masker was not presented, both the monaural and binaural features of FFRs to the chatter F0 were very similar to those of the tone h3 component. Also, when the noise masker was presented and the signal ITD was 1 ms, introducing an ITD disparity between the signal and masker caused an equal enhancement in the chatter-F0 amplitude and the tone-h3 amplitude.

However, when the noise masker was presented and the signal ITD was 0.1 ms, introducing an ITD disparity between the signal and masker caused a significantly larger enhancement of the chatter-F0 amplitude than the enhancement of the tone-h3 amplitude. Thus, although the chatter h2 and h3 components elicit less or no FFRs, they may make certain contributions to the ITD-disparity-induced enhancement of F0 FFRs against masking under the condition with the signal ITD of 0.1 ms, which is in the range of interaural traveling time of sound waves for rats. The specificity of FFRs to pain calls is an interesting issue for further investigation in the future.

### ***Cortically modulated binaural unmasking of FFRs***

In this study, when the noise masker was not presented, binaural interaction was not changed by bilaterally blocking area TE3 (Figure 5). However, when the noise masker was presented (Figure 8), the binaural unmasking effects under conditions of ipsilateral chatter leading were significantly enhanced by blocking excitatory glutamate transmissions in the area surrounding the AAC (area TE3), which is the cortical region sending direct axonal

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projections to the LA (Romanski and LeDoux 1993; Shi and Cassell 1997). The results suggest that the AAC plays a role in gating the binaural unmasking of FFRs in LA when the ipsilateral chatter leads the contralateral one.

What are the potential mechanisms underlying such cortical gating? It is well known that both principal (projection) neurons and inhibitory interneurons in LA receive excitatory afferents from both mMGN and AAC (Bauer and LeDoux 2004; Paré et al. 2004; Szinyei et al. 2000). Principal neurons interact with interneurons (Lang and Paré 1997, 1998; Mahanty and Sah 1998) and receive GABAergic inhibitory influence from interneurons (Bauer and LeDoux 2004; Lang and Paré 1997, 1998; Li et al. 1996; Szinyei et al. 2000). Thus interneurons in the LA may mediate the cortical gating.

The vast majority of excitatory mMGN-LA synapses occur on dendritic spines containing not only GluR1-3 subunits of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors but also the R1 subunit of N-methyl-D-aspartate receptors (NMDARs) (LeDoux et al. 1991; Farb and LeDoux 1997). Since dendritic spines occur mainly on LA principal neurons but not interneurons (McDonald 1982; Millhouse and de Olmos 1983; Nitecha and Ben-Ari 1987), the direct impact of mMGN afferents is stronger onto principal neurons than onto interneurons. Interestingly, NMDARs contribute mainly to excitatory transmissions at mMGN afferents, but to a lesser extent to those at AAC afferents (Li et al. 1995, 1996; Weisskopf and LeDoux 1999; Zinebi et al. 2001), and for glutamatergic inputs onto LA interneurons the contribution of NMDARs is very small or negligible (Mahanty and Sah 1998; Sah and de Armentia 2003). Thus although the AAC does excite the principal neurons in the LA, a large and direct impact of AAC afferents is on interneurons. Indeed, tetanic stimulation of the external capsule *in vitro*, which contains

axons projecting from AAC to LA, induces NMDAR-independent LTP in LA interneurons and augments inhibitory inputs to LA principal neurons (Mahanty and Sah 1998). In addition, electrical stimulation of the perirhinal and entorhinal cortical regions in anaesthetized cats produces much larger inhibitory effects on principal neurons than on interneurons in LA (Lang and Paré 1998). Specifically, the predominant response of principal neurons to high-current cortical stimuli is a large-amplitude hyperpolarization, while only a narrow range of low currents evokes orthodromic spikes. Moreover, short-latency excitatory responses of interneurons to cortical stimulation continue to increase with stimulation currents over a large range, and the excitatory response profile of interneurons corresponds with the inhibitory response profile of principal neurons.

Thus in the present study, the cortical top-down regulation of binaural unmasking of FFRs in LA may be mediated by inhibitory interneurons in LA. Since masking noise started earlier than the chatter (or the tone complex) and signal transportation in the MGB-AAC-LA pathway took longer than the mMGB-LA pathway, blocking AAC would reduce LA interneuron activity driven by masking noise and improve the salience of FFRs in LA. When masking noise was absent, FFRs to the chatter or the tone complex might be mainly (or completely) based on inputs from the mMGB, and blocking AAC in quiet did not substantially change FFRs to the chatter or the tone complex.

### ***Summary***

The major findings of this study are (1) signals of the rat's pain-call are precisely coded in rat amygdala in the form of FFRs that preserve the dominant F0 signal of the chatter; (2) the FFR F0 amplitude is stimulated-ear dependent, with an ipsilateral-input dominance,

which is also modulated by binaural inhibition, suggesting a functional relationship with spatial attention; (3) noise masking of LA FFRs can be reduced by binaural processing, which may reflect binaural unmasking in the central binaural pathways; (4) binaural unmasking is affected by top-down cortical gating.

Since the LA is known to be involved in eliciting defensive responses to threatening events, both binaural and cortical modulations of FFRs in rat LA are importantly associated with perception of life-threatening signals in noisy and even reverberant environments.

It should be noted that unlike the mouse pup wriggling calls, which also have vowel-like multi-harmonic structures and can reliably elicit maternal behavior of mother mice (Ehret and Riecke , 2002), the behavioural relevance of the rat chatter is still not clear. Particularly, behavioural and/or neural responses of a rat to hearing the chatter of another rat have not been reported in the literature. Thus the ecological significance of the chatter for rats is an important issue in future studies.

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## FIGURE CAPTIONS

**Figure 1.** (A): Spectrum of chatter showing a fundamental frequency (F0) at 2.1 kHz and two harmonics at 4.2 (h2) and 6.3 kHz (h3). (B): Spectrum of synthesized tone complex showing a F0 at 0.7 kHz and two harmonics at 2.1 (h3) and 3.5 kHz (h5).

**Figure 2.** Histological results of recording electrodes and injection cannulae in the 58 rats used in this study. Electrodes were precisely located within the LA area in 53 of 58 (left panel, filled circles), and cannulae were precisely located within area TE3 in 55 of 58 rats (right panel, filled circles). Incorrect locations of electrodes and cannulae are shown by open circles.

**Figure 3.** Typical response waveforms (panels A, B, C, D) and fast-Fourier spectral analyses of recorded FFRs (panels E, F, G, H) to contralateral and ipsilateral chatter or tone complex. Recording site contralateral to stimulated ear shows a marked onset response (panels A and C) but only moderate FFR amplitudes (panels E and G). Ipsilateral recording shows a much weaker onset response but a stronger sustained response in the original waveform (panels B and D) as well as increased F0 spectrum of chatter (panel F) and increased h3 spectrum of complex tone (panel H). The horizontal bar in panels A, B, C, D represents the duration of the stimulus.

**Figure 4.** Normalized tone-F0 amplitudes (panels A and D), tone-h3 amplitudes (panels B and E), and chatter-F0 amplitudes (panels C and F) in FFRs to signal under various monaural/binaural stimulation conditions before injection manipulation. The amplitude

evoked by contralateral-ear stimulation only (Stimulus Condition C) was used as the reference condition (amplitude = 1) for normalizing FFRs. Error bars in each panel are standard errors of the mean. Results are presented separately for ITD values of 0.1 (panels A, B, and C) and 1 ms (panels D, E, and F). Abbreviations: ITD, interaural time difference; I/C, binaural stimulation with ipsilateral signal (relative to recording site) leading contralateral; ST, simultaneously binaural stimulation; C/I, contralateral signal leading ipsilateral; I, signal at ipsilateral ear only; C, signal at contralateral ear only. \*  $P < 0.05$ , \*\*  $P < 0.01$  by one-way ANOVA and Bonferroni post hoc tests.

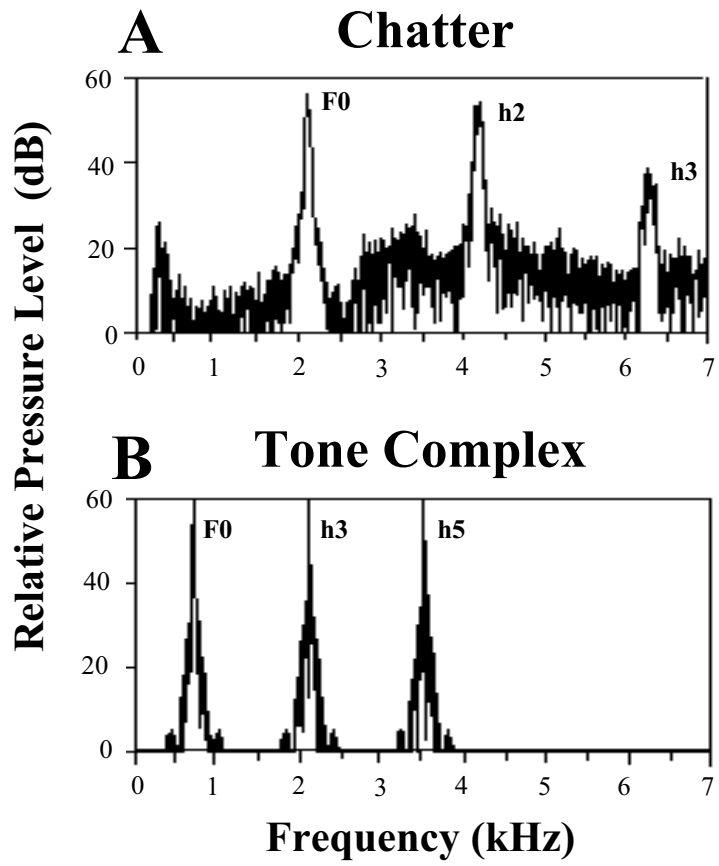
**Figure 5.** Normalized F0 spectral amplitudes of FFRs to chatter presented in quiet under various monaural/binaural stimulation conditions before (diagonal bars) and after (empty bars) either broad-spectrum glutamate receptor antagonist kynurenic acid (KYNA) (panels A and C) or Locke's solution (panels B and D) was bilaterally injected into temporal cortex area TE3. See Figure 4 caption for explanation of symbols and abbreviations.

**Figure 6.** Unmasking indices (UIs) when the tone complex (panels A, B, D, E) or chatter (panels C and F) was co-presented with correlated broadband noise to two ears with various ITD disparities  $|ITD_{S+N}|$  before injection. All UIs were computed under conditions when the ipsilateral signal led contralateral. UIs of tone-F0 amplitude (panels A and D) and tone-h3 amplitude (panels B and E) were computed separately. Numbers associated with each bar represent the  $|ITD_{S+N}|$ . \*  $P < 0.05$ , \*\*  $P < 0.01$  by one-way ANOVA and Bonferroni post hoc test assessing the significance of UIs under non-zero ITD disparity conditions compared with that under condition of  $|ITD_{S+N}| = 0$ .

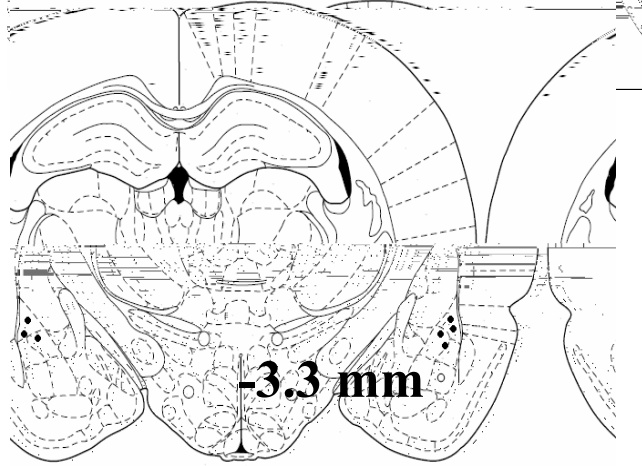
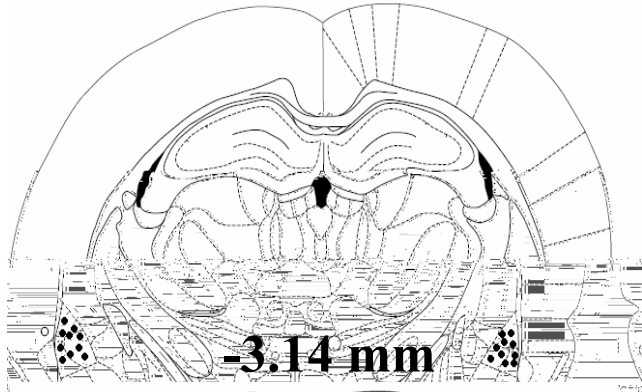
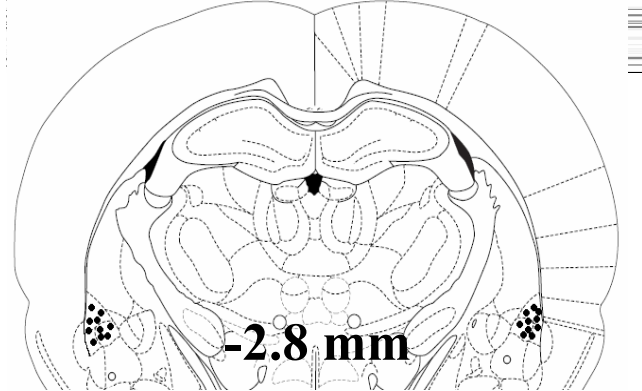
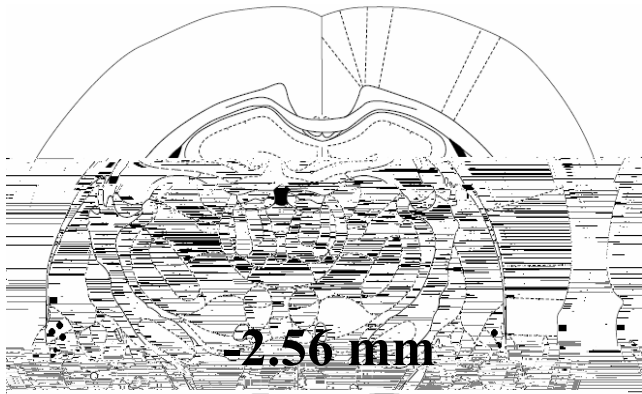


**Figure 7.** UIs when chatter and uncorrelated (independent) broadband masking noises were presented to two ears with various ITD disparities  $|ITD_{S+N}|$ . In this case, the binaural unmasking effect disappeared.

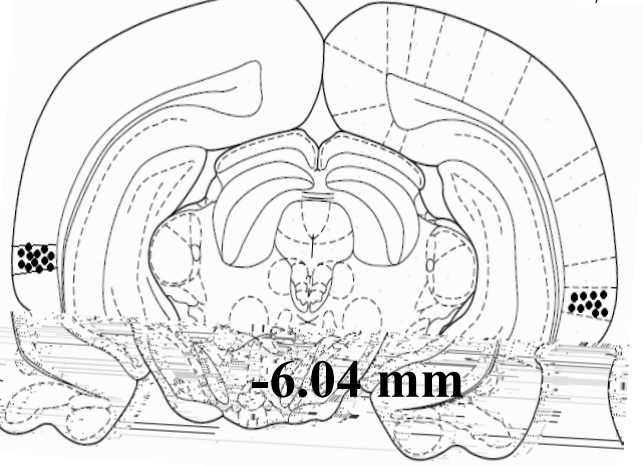
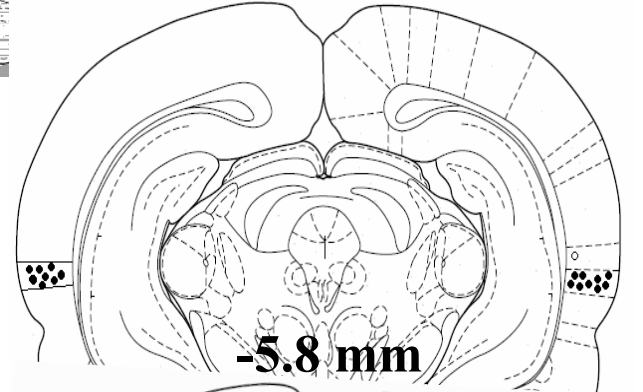
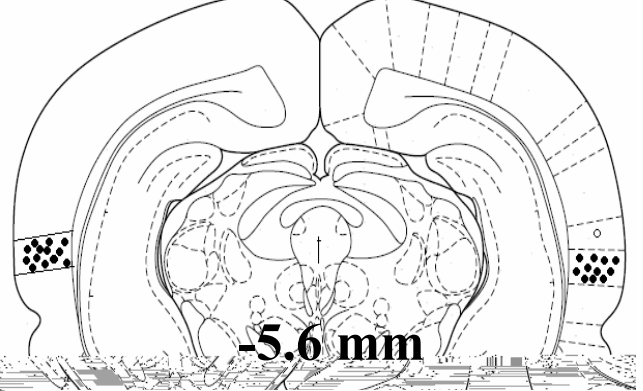
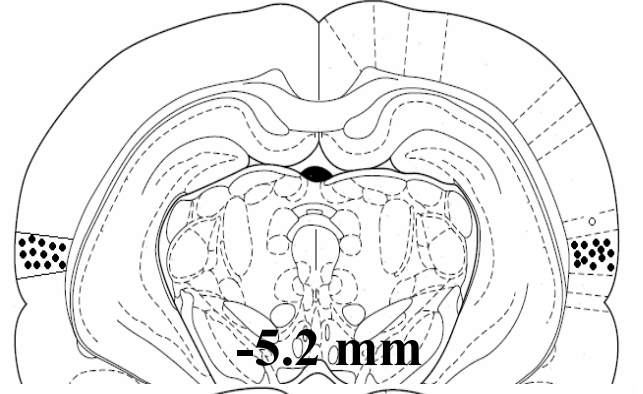
**Figure 8.** UIs when chatter and correlated broadband noise were presented to two ears with various ITD disparities  $|ITD_{S+N}|$  before (grey bars) and after (diagonal bars) temporal area TE3 was bilaterally injected with KYNA (panels A and C) or Locke's solution (panels B and D). \*  $P < 0.05$ , \*\*  $P < 0.01$  by paired-samples t-test assessing the agent effect on UIs.

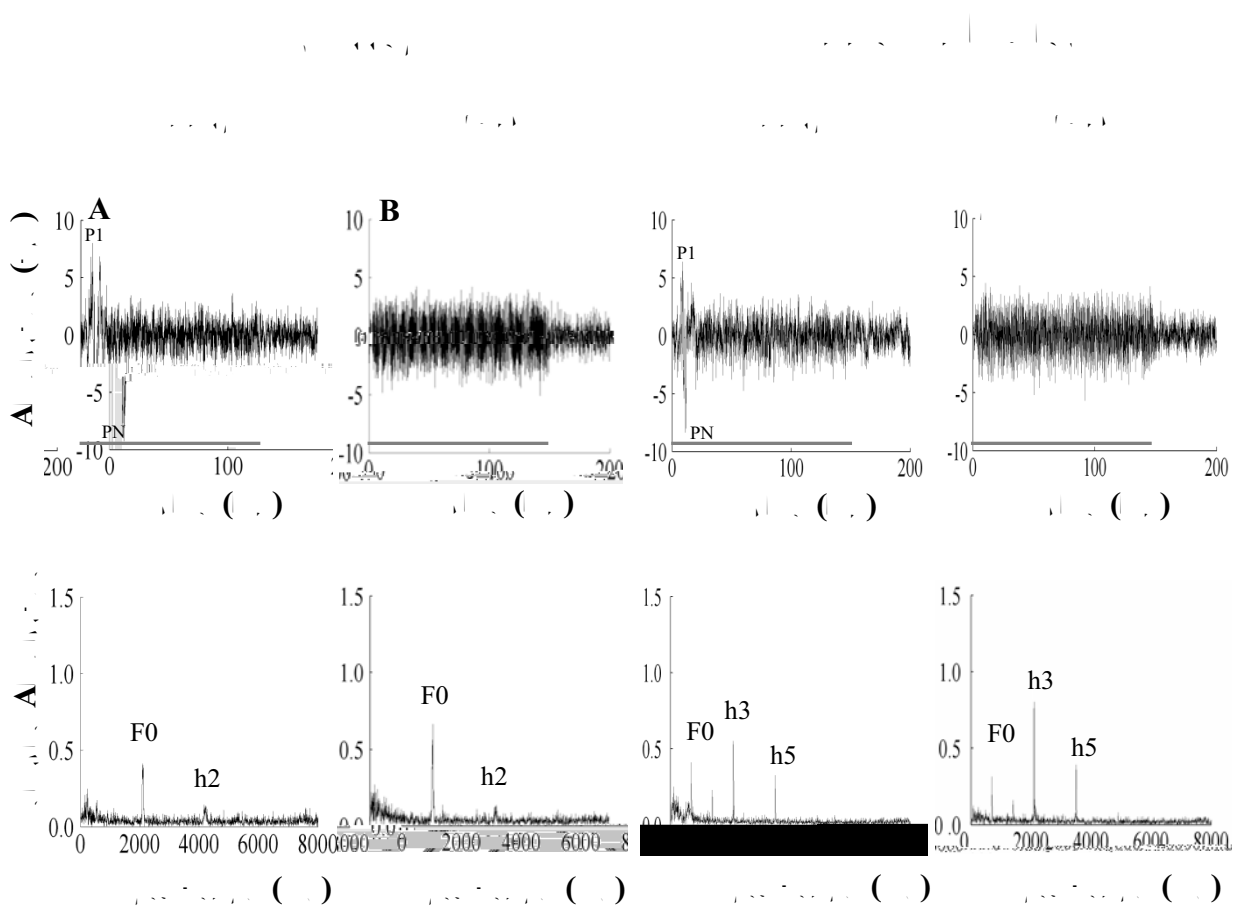


**LA**

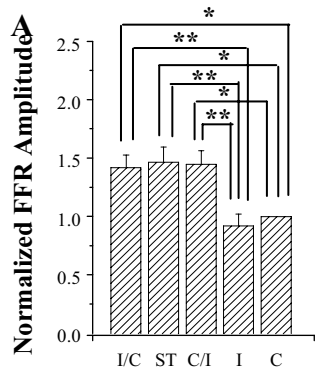


**TE3**



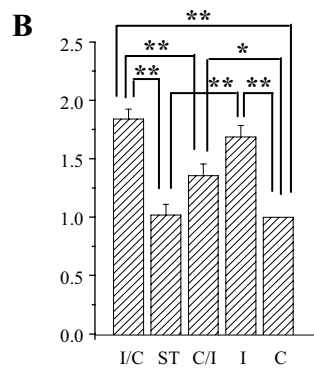


**Tone-F0**

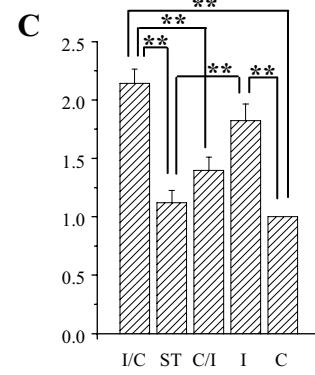


**Tone-h3**

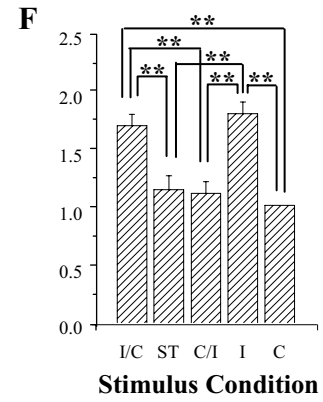
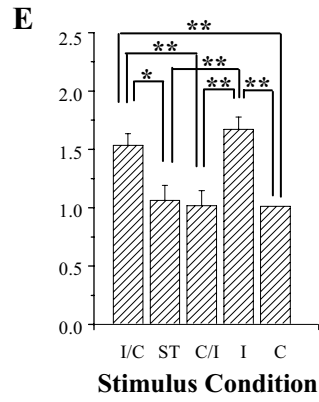
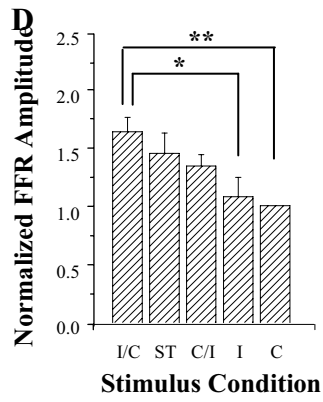
**ITD = 0.1 ms**



**Chatter-F0**



**ITD = 1 ms**

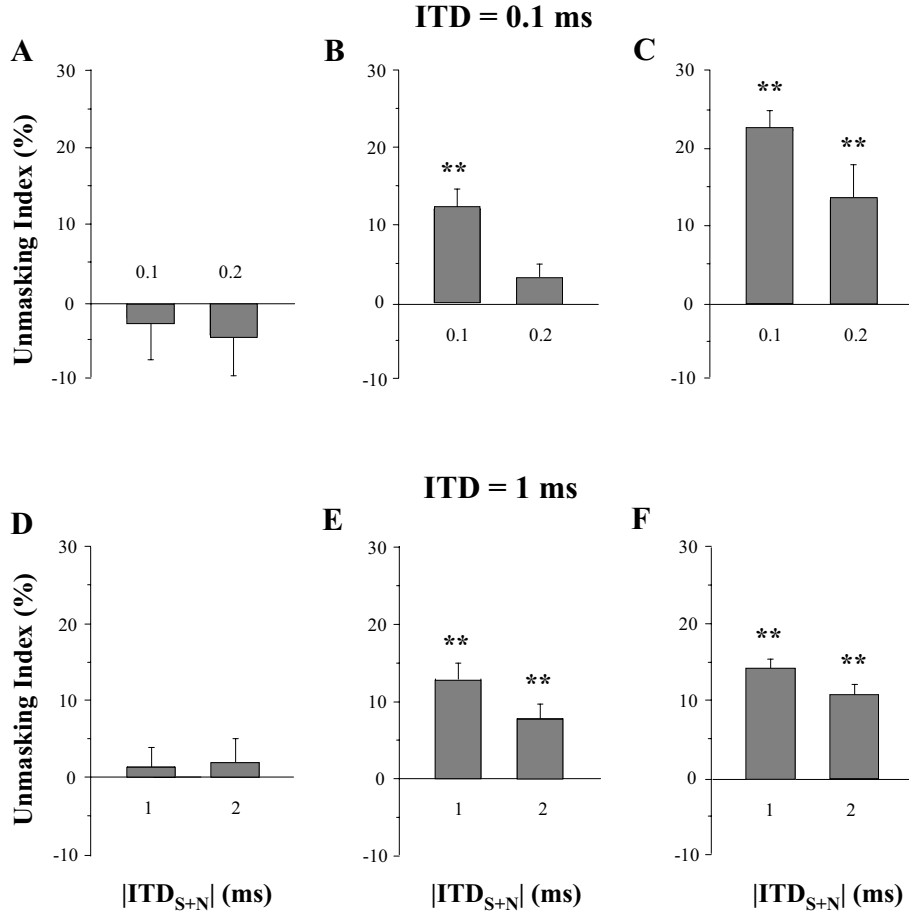




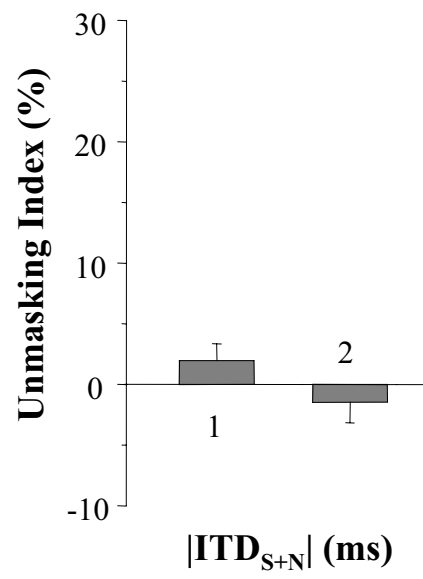
**Tone-F0**

**Tone-h3**

**Chatter-F0**



## Noise-Control

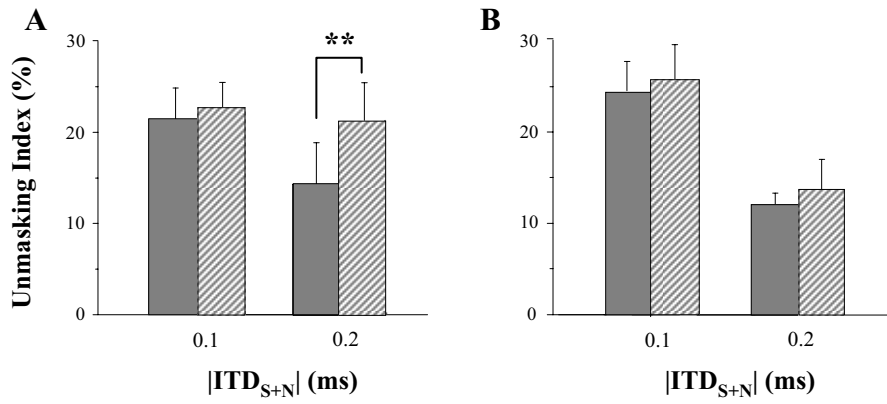




### KYNA

### Locke's

Chatter ITD = 0.1 ms



Chatter ITD = 1 ms

