

# PERCEIVED TARGET–MASKER SEPARATION UNMASKS RESPONSES OF LATERAL AMYGDALA TO THE EMOTIONALLY CONDITIONED TARGET SOUNDS IN AWAKE RATS

Y. DU, Q. WANG, Y. ZHANG, X. WU AND L. LI\*

Department of Psychology, College of Life Sciences, Speech and Hearing Research Center, Key Laboratory on Machine Perception (Ministry of Education), Peking University, Beijing 100871, China

**Abstract**—In a (simulated) reverberant environment, both human listeners and laboratory rats are able to perceptually integrate the direct wave of a sound source with the reflections of the source, leading to a fused image as coming from the location around the source (the precedence effect). This perceptual grouping effect produces perceived spatial separation between sound sources and facilitates selective attention to the target source. However, the neural correlates of the unmasking effects of perceived spatial separation have not been reported in the literature. The lateral nucleus of the amygdala (LA) is critical for processing ecologically salient sensory signals (e.g., threatening sounds) and mediating auditory fear conditioning. LA neuronal responses to a sound increase if the sound is fear conditioned. This study investigated whether in awake rats the perceptual fusion-induced separation between a fear-conditioned target sound and a noise masker enhances LA responses to the target. The results show that frequency-following responses (FFRs, i.e., sustained potentials based on phase-locked firing of neuron populations to periodical sound waveforms) recorded in the LA to a tone-complex, which was masked by a wideband noise, were enhanced after the tone-complex became fear conditioned. More importantly, the fear-conditioned tone-complex, but not the pseudo-conditioned tone-complex, elicited further larger LA FFRs when it was perceived as separated from the masker than when it was perceived as co-located with the masker. The results suggest that in the LA there exists a neural correlate of selective attention to ecologically significant sounds with a high degree of stimulus specificity. © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.

**Key words:** amygdala, fear conditioning, attention, precedence effect, frequency-following responses.

\*Corresponding author. Address: Department of Psychology, Peking University, Beijing 100871, China. Tel: +86-10-6275-6804; fax: +86-10-6276-1081.

E-mail address: liangli@pku.edu.cn (L. Li).

**Abbreviations:** ANOVA, analysis of variance; BOLD, blood oxygen level-dependent; CI, contralateral loudspeaker led ipsilateral loudspeaker; CS, conditioned stimulus; FFR, frequency-following response; FFT, fast Fourier transform; IC, ipsilateral loudspeaker led contralateral loudspeaker; LA, lateral nucleus of the amygdala; MEG, magnetoencephalography; PPC, posterior parietal cortex; PPI, prepulse inhibition; SNR, signal-to-noise ratio; ST, simultaneously bilateral stimulation; US, unconditioned stimulus.

## INTRODUCTION

The amygdala mediates fear-related attention toward the most salient signal, such as a threat, under stressful circumstance (e.g., Meck and MacDonald, 2007) and forms auditory fear conditioning (for reviews see Phelps and LeDoux, 2005; Maren, 2011). The lateral nucleus of the amygdala (LA) occupies the initial stage receiving inputs from the auditory thalamus and associate auditory cortex (LeDoux et al., 1990; Romanski and LeDoux, 1993). Previous animal studies have shown that responses of LA neurons to a sound markedly increase after the sound becomes fear conditioned (Maren et al., 1991; Quirk et al., 1995; Collins and Paré, 2000; Maren, 2000).

There has been extensive debate regarding whether amygdala responses to emotionally salient stimuli are either automatic without attention (e.g., Whalen et al., 1998; Vuilleumier et al., 2001; Anderson et al., 2003; Pasley et al., 2004) or attentionally modulated (e.g., Pessoa et al., 2002; Bishop et al., 2007; Straube et al., 2007; Mothes-Lasch et al., 2011; for a critical review see Brosch and Wieser, 2011). For example, in the Mothes-Lasch et al. (2011) study, brain blood oxygen level-dependent (BOLD) responses to angry speech prosody against those to emotionally neutral prosody were investigated using a cross-modal distraction paradigm in which both the acoustic and visual stimuli were presented simultaneously. The results indicate the higher activation to angry prosody versus neutral prosody in the left amygdala, left insula, and bilateral superior temporal cortex when participants attended to the gender of the speaker's voice (the auditory attributes). However, this effect was completely absent when participants' attention was directed to the non-emotional visual stimulus, suggesting that the shift of attention to the visual distracter reduces the acoustically anger-prosody-induced activation in the amygdala and the cortical regions. To further understand the relationship between amygdala activity and selective attention to emotional stimuli, it is necessary to examine whether LA responses in laboratory animals to an ecologically significant sound can be enhanced when the attention allocated to the ecologically significant sound is further facilitated.

What is the suitable experimental paradigm that modulates auditory spatial attention without affecting bottom-up sensory-input impacts in both humans and laboratory animals? Masking of a target sound can be

reduced if a spatial separation is introduced between the target and the masker. The spatial unmasking is caused by a combination of three effects: (1) the head-shadowing effect (which improves the signal-to-masker ratio in sound-pressure level at the ear near the target), (2) the interaural-time-difference-disparity effect (which enhances auditory neuron responses to the target sound), and (3) the psychological effect (which facilitates spatial attention to the target). Interestingly, when the listening environment is reverberant, in which a sound source induces numerous reflections bouncing from surfaces, both the unmasking effect of head shadowing and that of interaural-time-difference disparity are limited or even abolished, but the psychological unmasking caused by perceived spatial separation between the target and masker is still effective (Koehnke and Besing, 1996; Freyman et al., 1999; Zurek et al., 2004; Kidd et al., 2005). Thus, introducing a (simulated) reverberant listening condition can be used for isolating the psychologically unmasking effect. This unmasking effect is closely associated with the auditory precedence effect (see below).

What is the precedence effect? What is the role of the precedence effect in noisy, reverberant environments? In a (simulated) reverberant environment, to distinguish signals from various sources and particularly recognize the target source, listeners need to not only perceptually integrate the direct wave with the reflections of the target source (Huang et al., 2008, 2009) but also to perceptually integrate the direct wave with the reflections of the masking source (Brungart et al., 2005; Rakerd et al., 2006). More specifically, when the delay between a leading sound (such as the direct wave from a sound source) and a correlated lagging sound (such as a reflection of the direct wave) is sufficiently short, attributes of the lagging sound are perceptually captured by the leading sound (Li et al., 2005), causing a single fused sound image as coming from a location near the leading source (*the precedence effect*, see Wallach et al., 1949; Zurek, 1980; Freyman et al., 1991; Litovsky et al., 1999; Huang et al., 2011). This perceptual fusion is able to produce perceived spatial separation between uncorrelated sound sources. For example, when both the target and masker are presented by a loudspeaker to the listener's left and by another loudspeaker to the listener's right, the perceived location of the target and that of the masker can be manipulated by changing the inter-loudspeaker time interval for the target and that for the masker (Li et al., 2004). More in detail, for both the target and masker, when the sound onset of the right loudspeaker leads that of the left loudspeaker by a short time (e.g., 3 ms), both a single target image and a single masker image are perceived by human listeners as coming from the right loudspeaker. However, if the onset delay between the two loudspeakers is reversed only for the masker, the target is still perceived as coming from the right loudspeaker but the masker is perceived as coming from the left loudspeaker. The perceived co-location and perceived separation are based on perceptual integration of correlated sound waves delivered from each of the two loudspeakers. It has been

confirmed that perceived target–masker spatial separation facilitates the listener's selective attention to target signals and significantly improves recognition of target signals (Freyman et al., 1999; Li et al., 2004; Wu et al., 2005; Rakerd et al., 2006; Huang et al., 2008, 2009). It should be emphasized that when the two loudspeakers are symmetrical to the listener, a change between the perceived co-location and the perceived separation alters neither the target-to-masker ratio in sound pressure level at each ear nor the stimulus-image compactness/diffusiveness (Li et al., 2004).

Does the precedence effect occur in laboratory animals? It has been documented that in behaving rats the precedence effect can be demonstrated and the 1-ms inter-sound delay is within the range for producing perceptual fusion (e.g., Kelly, 1974; Hoeffding and Harrison, 1979). The precedence effect has also been demonstrated in other laboratory species such as barn owls (Spitzer and Takahashi, 2006). Particularly, the precedence-effect-induced separation also modulates body responses to the target sound in laboratory rats (see below).

How to use the precedence effect to establish a simple and efficient animal behavioral model for studying selective spatial attention? The startle reflex, the whole-body reflexive response to sudden and intense sensory stimuli (Koch, 1999; Yeomans et al., 2002), can disrupt cognitive/behavioral performances (Hoffman and Overman, 1971; Foss et al., 1989). Prepulse inhibition (PPI) of startle is the suppression of the startle reflex when a weaker sensory stimulus (the prepulse) shortly precedes the intensive startling stimulus (Hoffman and Searle, 1965; Hoffman and Ison, 1980). In rats, PPI is enhanced when the prepulse stimulus becomes fear conditioned (Huang et al., 2007; Zou et al., 2007; Li et al., 2008; Du et al., 2009b, 2010, 2011b), suggesting that selective attention to the ecologically salient prepulse stimulus facilitates PPI (Li et al., 2009). Moreover, when the prepulse is masked by a broadband noise, the conditioning-induced PPI enhancement in rats can be further increased by precedence-effect-induced perceived spatial separation between the fear-conditioned prepulse stimulus and the masking noise without affecting bottom-up sensory-input impacts (Du et al., 2009b, 2010, 2011b), suggesting that the perceived spatial separation facilitates selective attention to the conditioned prepulse and improves the conditioned-prepulse-induced PPI.

As mentioned before, responses of LA neurons to a sound increase if the sound is fear conditioned (Maren et al., 1991; Quirk et al., 1995; Collins and Paré, 2000; Maren, 2000). This study extended this line of research by investigating whether a target–masker perceived spatial separation (induced by the precedence effect) affects LA responses to the target sound before and after the sound is fear conditioned.

When a target sound and one or more masking sounds occur simultaneously, both the target and masker(s) elicit responses of auditory neurons. How can the auditory responses specific to the target be isolated? Frequency-following responses (FFRs), which

are sustained potentials based on phase-locked firing of neuron populations to low-to-medium-frequency periodical sound waveforms (Worden and Marsh, 1968), are particularly useful for studying attentional modulation of stimulus-specific auditory responses when one or more sound sources are presented simultaneously (Du et al., 2011a). In laboratory rats, FFRs to tone-complexes can be directly recorded within the LA (Du et al., 2009a) and the auditory midbrain inferior colliculus (Ping et al., 2008; Du et al., 2009c). The purpose of this study was to investigate whether LA FFRs in awake rats to a masked tone-complex can be unmasked (enhanced) by a perceived spatial separation between the tone-complex and the masker, either before or after the tone-complex is fear conditioned.

## EXPERIMENTAL PROCEDURES

### Animal preparation

Fourteen young-adult male Sprague–Dawley rats (11 weeks, 280–300 g), which were treated in accordance with the Guidelines of the Beijing Laboratory Animal Center, and the Policies on the Use of Animals and Humans in Neuroscience Research (Society for Neuroscience, 2006), first received electrode-implantation surgery. Briefly, one stainless steel recording electrode (10–20 k $\Omega$ ) insulated by a silicon tube (0.3 mm in diameter) except the 0.25-mm diameter tip (Zheng et al., 2008) was aimed at unilateral LA (with left–right counterbalance across rats) in each of the 10% chloral hydrate (400 mg/kg, i.p.) anesthetized rats. Referenced to Bregma, the stereotaxic coordinates were: anteroposterior,  $-3.1$  mm; mediolateral,  $\pm 5.2$  mm; depth,  $-7.8$  mm. Two micro-screws embedded into the skull served as the reference and ground electrodes. Electrodes were mounted on the skull with dental cement and connected to a male adaptor. Rats were given one-week for recovery in a room with  $24 \pm 2$  °C temperature and a 12 h light/dark cycle, with food and water available *ad libitum*.

### Stimuli

A 50-ms lower-frequency-harmonic (1.3, 2.6, and 3.9 kHz) tone-complex and a 50-ms higher-frequency-harmonic (2.3, 4.6, and 6.9 kHz) tone-complex were used as the target stimuli. A 600-ms (including 5-ms linear onset/offset ramps) segment of broadband noise (0–10 kHz) starting 300 ms before the tone-complex onset was used as the masker. All sound waves were processed by a TDT System II (Tucker-Davis Technologies, FL, USA), and presented by two spatially separated (i.e., left and right) TDT loudspeakers in the frontal field with a 90° angle and 14.1 cm away from the rat's head position. Calibrated by a sound level meter (Brüel & Kjær, B&K, Type 2230), the intensity of the tone-complex under quiet conditions was fixed at 56 dB SPL when each loudspeaker was played alone. Under noise-masking conditions, the intensity of the single-source masking noise was held constant at 60 dB SPL while the whole-spectrum intensity of the tone-complex was adjusted to produce 4 signal-to-noise ratios (SNRs):  $-8$ ,  $-4$ ,  $0$ , and  $4$  dB.

### Physiological recordings

Recordings were conducted in a sound-attenuating chamber. Facing the frontal field, the awake rat was placed in a one-piece-dress-like holder, which was hung in the air. The specially designed holder, which was made of soft cloth, limited the rat's body movement but left rat's head, limbs, and tail

unrestrained. The electrode male adaptor on the rat's head was then connected to a female adaptor that was connected to a TDT DB4 amplifier. Recordings started 30 min after the adaptation (see below) when the rat exhibited minimal body movement. Trials contaminated by artifacts (e.g., myoelectricity) were excluded from the analyses.

Neural potentials were recorded for an 80-ms epoch (10 ms before tone-complex onset to 20 ms after tone-complex offset) for FFRs in quiet and a 630-ms epoch (10 ms before noise onset to 20 ms after noise offset) for FFRs in noise, amplified 1000 times, filtered through 200–10000 Hz band pass, and averaged 50 times per condition.

### Procedures

Each rat underwent a 2-day testing procedure. On Day 1, rats first adapted to the restraining clothes in the recording chamber for 30 min. And then the following stimuli were presented: (1) unilateral (left loudspeaker only, right loudspeaker only) tone-complex in quiet, (2) bilateral tone-complex in quiet with the following inter-loudspeaker onset delay:  $-1$  ms (right loudspeaker leading),  $0$  ms, and  $+1$  ms (left loudspeaker leading), and (3) bilateral tone-complex in masking noise (more detail see below).

Under noise-masking conditions, both the tone-complex and the masker were presented by each of the two loudspeakers. The inter-loudspeaker onset delay for the tone-complex was  $-1$  ms (right leading) or  $+1$  ms (left leading). Due to the precedence effect, we assume that a single fused tone-complex image would be perceived at the right loudspeaker in some trials (when right loudspeaker led) and at the left loudspeaker in other trials (when left loudspeaker led). The inter-loudspeaker onset delay for the noise masker was also  $-1$  or  $+1$  ms. Thus, there were two types of perceived spatial relationships between the tone-complex and the masker: perceptual co-location (when the tone-complex and masker shared the same leading loudspeaker) and perceptual separation (when the tone-complex and masker had different leading loudspeakers). In addition, there were two types of hemispherical relationships between the side (left or right) of the recording electrode and the side (left or right) of the perceived location of tone-complex: ipsilateral and contralateral. Thus, there were 2 (stimulus type: lower-frequency-harmonic, and higher-frequency-harmonic)  $\times$  4 (SNR)  $\times$  2 (perceptual spatial relationship between tone-complex and masker: co-location, and separation)  $\times$  2 (perceptual hemispherical relationship between tone-complex image and recording side: ipsilateral, and contralateral) conditions. The presentation order of the 32 conditions was arranged with a pseudo-random manner with each condition repetitively presented 50 times. The stimulus onset asynchrony was 1000 ms for the tone-complex both in quiet and in noise.

Also on Day 1, after FFR recordings, rats underwent a discriminative fear conditioning procedure (fear conditioning of only one tone-complex and conditioning-control manipulation (pseudo-conditioning) of the other tone-complex). For a rat, the conditioned stimulus (CS+) was one of the two tone-complexes and the pseudo-CS+ was the other tone-complex. Each of the two types of CSs was delivered by the two loudspeakers with balanced left–right leading, and the unconditioned stimulus (US) was a 6-mA rectangular-pulse (duration = 3 ms) of footshock (generated by a Grass S-88 stimulator) delivered to the rear paw (Zou et al., 2007; Du et al., 2011b).

During the fear-conditioning manipulation, 10 temporally paired combinations of the US and the CS+ were presented every 30 s (US started 3 ms before CS+ ending, and co-terminated with CS+). During the conditioning-control manipulation, 10 temporally unpaired (random) combinations of the US and the pseudo-CS+ were presented every 30 s. For half of the 14 rats, the lower-frequency-harmonic tone-complex

was used as the CS+ and the higher-frequency one was used as the pseudo-CS+, while the other half received the contrary manipulations.

On Day 2 (24 h after discriminative conditioning), FFRs were recorded again with the recording procedure described above.

## Data analyses

For FFRs to the contralaterally (unilaterally) presented tone-complex in quiet, a 1-kHz low-pass filter was applied to smooth the potential, and the peak-potential response latency was determined by measuring the time interval between the sound onset and the first positive peak of the response waveform.

For FFRs in quiet, fast Fourier transform (FFT) was performed for each unfiltered FFR waveform. The spectral peak amplitude of a 100-Hz-wide band centered at either 1.3 kHz (the fundamental frequency (F0) of the lower-frequency tone-complex) or 2.3 kHz (the F0 of the higher-frequency tone-complex) was labeled as the FFR F0 amplitude.

For FFRs in noise, FFT at each condition was performed for a period from the tone-complex onset to 10 ms after the tone-complex offset, which contained both signal information and masker information. The spectral peak amplitude of a 100-Hz-wide band centered at either 1.3 kHz (for the lower-frequency tone-complex) or 2.3 kHz (for the higher-frequency tone-complex) was labeled as the FFR F0 amplitude of signal in noise-masking conditions (AMPs + n). On the other hand, the mean spectral amplitude of two 100-Hz-wide sidebands centered at 1.2 and 1.4 kHz (for the lower-frequency tone-complex) or 2.2 and 2.4 kHz (for the higher-frequency tone-complex) was defined as the amplitude of noise (AMPn). The response SNR at each stimulus SNR was defined as the following:

$$\text{Response SNR} = 20\lg(\text{Amp}_{s+n}/\text{Amp}_n)$$

## Histology

Rats were killed with an overdose of chloral hydrate after the experiment. Lesion marks were made via recording electrodes by an anodal DC current (500  $\mu$ A for 10 s). Brains were stored in 10% formalin with 30% sucrose, then sectioned at 50  $\mu$ m in the frontal plane in a cryostat ( $-20^\circ\text{C}$ ). Sections were examined to determine the locations of recording electrodes.

# RESULTS

## Histology

According to histological examination (Fig. 1), recording electrodes were precisely located in the LA area in seven rats with the left-inserted electrode and in six rats with the right-inserted electrode. The electrode placed in left LA area in one rat (open triangle) displayed over-threshold impedance. Rats with either electrode misplacement or over-threshold impedance were removed from data analyses, leaving valid data from 12 rats.

## LA FFRs were selectively enhanced by fear conditioning

Before the fear conditioning/conditioning-control manipulation, both the lower-frequency-harmonic tone-complex and the higher-frequency-harmonic tone-complex elicited reliable FFRs in the LA either in quiet or under the noise-masking condition. For example, the

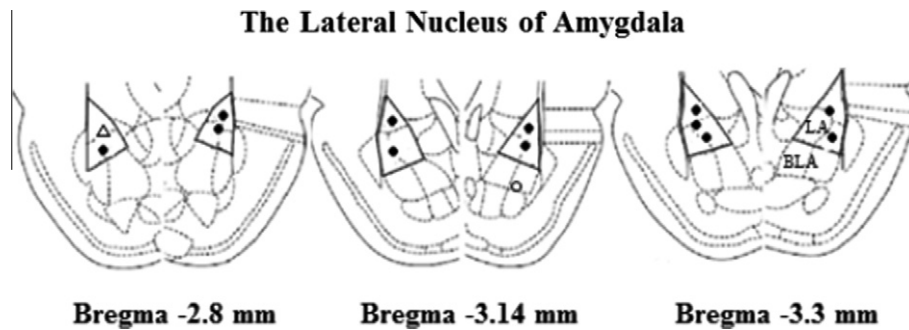
lower-frequency tone-complex was able to elicit FFRs either for each of its three harmonic components in quiet (Fig. 2a, b) or for the two low-frequency components (1.3 and 2.6 kHz) under noise masking even though the onset field-potential response to the tone-complex was undetectable (Fig. 2c, d).

After the fear conditioning/conditioning-control manipulation, both the latency of LA evoked potentials and spectral power of LA FFRs changed with the stimulus specificity. For the latency (defined by the first positive peak of LA evoked potentials and averaged across rats and tone-complex types), when a tone-complex contralateral to the recording side was presented without noise masker and used as the CS+, it was 8.27 ms (SD = 0.19 ms) before the conditioning/conditioning-control manipulation and 7.75 ms (SD = 0.41 ms) after the manipulation. The latency difference was significant ( $t_{11} = 5.616$ ,  $p < 0.001$ , paired- $t$  test). However, when the tone-complex served as the pseudo-CS+, it was 8.21 ms (SD = 0.16 ms) before the conditioning/conditioning-control manipulation and 8.13 ms (SD = 0.31 ms) after the manipulation. The latency difference was not significant ( $t_{11} = 1.36$ ,  $p > 0.05$ ). And the latency around 8.2 ms before fear conditioning is consistent with our previous report that it is  $6.29 \pm 0.46$  ms in the rat IC (Du et al., 2009c) and  $8.03 \pm 0.68$  ms in the rat LA (Du et al., 2009a).

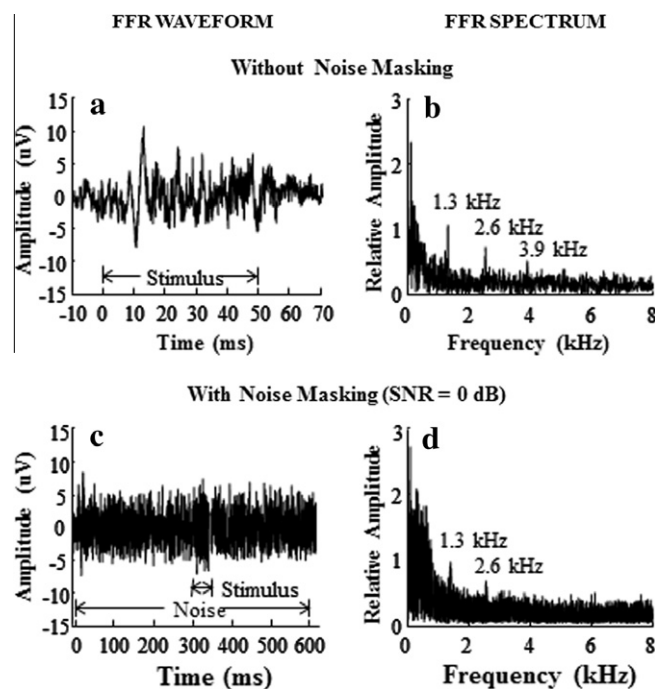
For the FFR amplitude, as shown by Fig. 3, under the condition without noise masking, the amplitude of LA FFRs to the F0 of the CS+ was significantly enhanced ( $F_{(1,11)} = 10.09$ ,  $p < 0.01$ , within-subject (ANOVA, analysis of variance)) whenever it was presented bilaterally (IC, ipsilateral loudspeaker led contralateral loudspeaker; ST, simultaneously presented; CI, contralateral loudspeaker led ipsilateral loudspeaker) or unilaterally (I, ipsilateral only; C, contralateral only). However, the F0 amplitude of LA FFRs to the pseudo-CS+ was not affected by the manipulation under each of the stimulation conditions (i.e., IC, CI, ST, I, C) ( $F_{(1,11)} < 1$ ,  $p > 0.05$ , within-subject ANOVA).

When the tone-complexes were co-presented with the noise masker, as Fig. 4 shows, LA FFRs (represented by response SNR for the F0 component) to the CS+ after the conditioning/conditioning-control manipulation (Fig. 4c, d) were larger than those before the manipulation (Fig. 4a, b). However, the enhancement of LA FFRs did not occur for the pseudo-CS+ (Fig. 4e, f). Also, the CS+ elicited stronger LA FFRs than the pseudo-CS+ after the conditioning/conditioning-control manipulation, regardless of whether the CS+ was perceived ipsilaterally (Fig. 4c vs. e) or contralaterally (Fig. 4d vs. f) to the side of recording electrode.

Following a combination of FFRs to the perceived ipsilateral tone-complex and FFRs to the perceived contralateral tone-complex after the fear conditioning/conditioning-control manipulation, 2 (stimulus type: CS+, pseudo-CS+)  $\times$  4 (stimulus SNR) repeated-measures ANOVAs show that under the perceived CS+ masker co-location condition (full lines in Fig. 4c–f), LA FFRs to the CS+ were significantly larger than those to the pseudo-CS+ ( $F_{(1,23)} = 10.85$ ,  $p < 0.01$ ) without



**Fig. 1.** Locations of electrodes aimed to the lateral nucleus of the amygdala (LA) in 14 rats. The electrode was correctly inserted within the LA area in 13 rats (12 filled circles and one open triangle), but misplaced in one rat (open circle). One rat with correct electrode placement displayed over-threshold impedance (open triangle).



**Fig. 2.** Typical FFR waveforms and FFR spectra to the lower-frequency-harmonic (1.3, 2.6, and 3.9 kHz) tone-complex. (a and b) FFR waveform and spectrum to the tone-complex in quiet; (c and d) FFR waveform and spectrum to the tone-complex under noise masking.

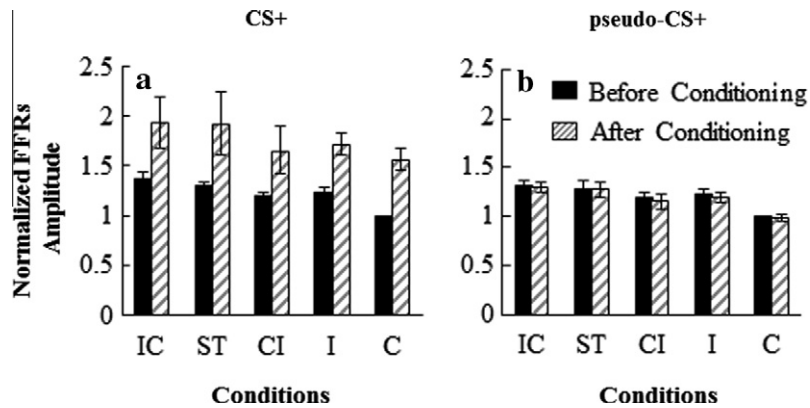
significant interaction between CS type and SNR ( $F_{(3,69)} < 1$ ,  $p > 0.05$ ). Under the perceived CS-masker separation condition (dashed lines in Fig. 4c–f), the CS type interacted significantly with SNR ( $F_{(3,69)} = 4.84$ ,  $p < 0.01$ ), and further paired *t*-tests indicate significantly larger LA FFRs to the CS+ than those to the pseudo-CS+ at each of the four SNRs (all  $t_{(23)} > 4.7$ ,  $p < 0.001$ ). Thus, although the CS+ and the pseudo-CS+ were homogeneous (each CS type contains both lower-frequency tone-complex and higher-frequency tone-complex), FFRs to the CS+ were significantly larger than FFRs to the pseudo-CS+ after the fear conditioning/conditioning-control manipulation.

#### Effects of perceptual spatial separation on LA FFRs before conditioning

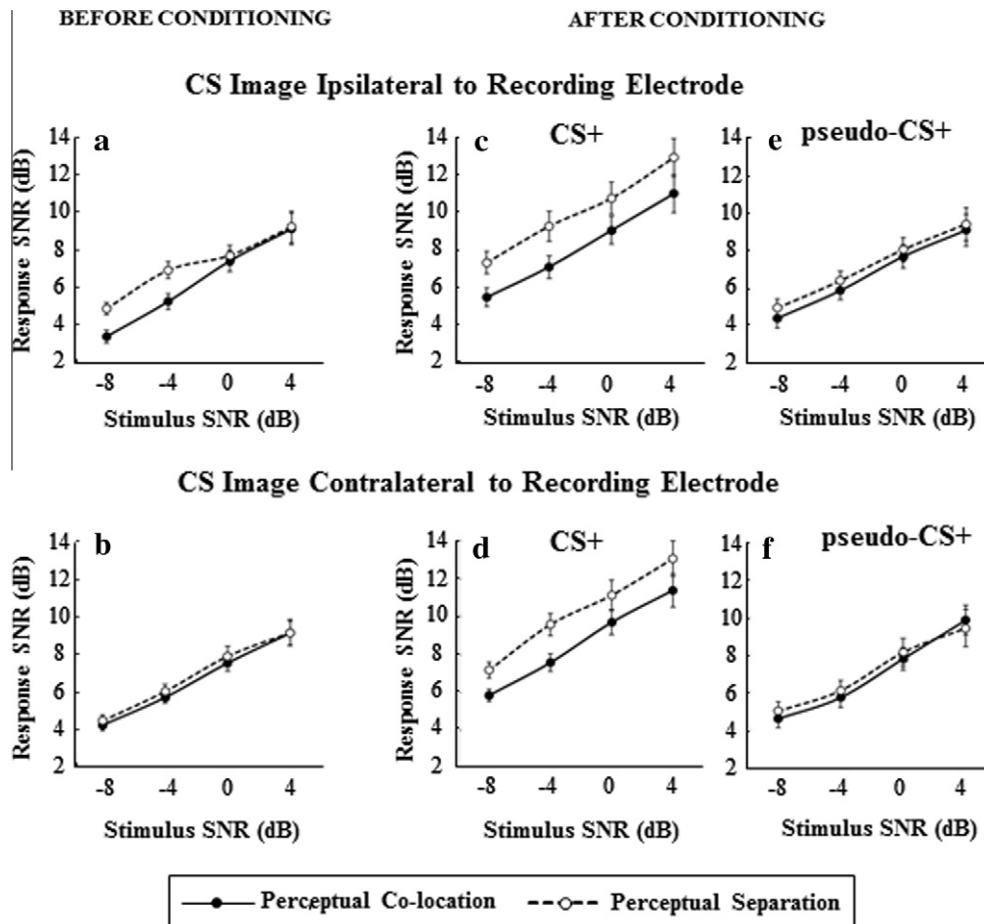
To simplify the description and statistical analyses of the results before the conditioning/conditioning-control

manipulation, the response SNR data for LA FFRs were combined across the two types (lower- and higher-frequencies) of the tone-complex. Fig. 4a, b show that the spatial separation effect (dashed line vs. solid line) appears to be determined by the interaction between stimulus SNR and laterality relationship between the electrode and the CS image: The marked separation effect occurred only at low SNRs when the recording side was ipsilateral to the side of perceived position of the tone-complex.

For FFRs to the ipsilateral CS image (Fig. 4a), a 2 (separation type: co-location, separation)  $\times$  4 (stimulus SNR) repeated-measures ANOVA reveals a significant interaction between separation type and SNR ( $F_{(3,69)} = 24.55$ ,  $p < 0.001$ ). Further paired *t*-tests show that FFRs to the CS under the separation condition were remarkably larger than those under the co-location condition when the stimulus SNR was either  $-8$  or



**Fig. 3.** Normalized F0 amplitudes of FFRs to the CS+ (a) and those to the pseudo-CS+ (b) in quiet under various unilateral/bilateral stimulation conditions before (black bars) and after (diagonal bars) the conditioning/conditioning-control manipulation. The amplitude evoked by contralateral stimulation only (condition C) was used as the reference condition (amplitude = 1) for normalization. Error bars in each panel are standard errors of the mean (SE). *Abbreviations:* IC, ipsilateral loudspeaker (relative to recording site) led contralateral; ST, simultaneously bilateral stimulation; CI, contralateral loudspeaker led ipsilateral; I, ipsilateral loudspeaker only; C, contralateral loudspeaker only.



**Fig. 4.** Response SNRs of LA FFRs to the CS (CS+, pseudo-CS+) as a function of the stimulus SNR under the condition of perceptual CS-masker spatial separation (dashed lines) or the condition of perceptual co-location (solid lines) either before (a, b) or after (c–f) the fear conditioning/conditioning-control manipulation.

–4 dB (both  $t_{(23)} > 9.38$ ,  $p < 0.001$ ) but not when the stimulus SNR was 0 or 4 dB (both  $t_{(23)} < 1.9$ ,  $p > 0.05$ ). However, for FFRs to the contralateral CS image (Fig. 4b), neither the interaction between separation

type and SNR ( $F_{(3,69)} < 1$ ,  $p > 0.05$ ) nor the main effect of separation type ( $F_{(1,23)} = 3.25$ ,  $p > 0.05$ ) was significant. The main effect of SNR was significant ( $F_{(3,69)} = 74.34$ ,  $p < 0.001$ ).

### Spatial separation enhanced LA FFRs to the CS+ but not the pseudo-CS+ after the fear conditioning/conditioning-control manipulation

As indicated by Fig. 4c–f, after the fear conditioning/conditioning-control manipulation, the spatial separation effect on LA FFRs was affected by the CS type (CS+ or pseudo-CS+). Thus, descriptions and statistical analyses of the results were conducted for FFRs to the CS+ and for FFRs to the pseudo-CS+, respectively.

Obviously, perceptual separation remarkably enhanced FFRs to the CS+ (Fig. 4c, d, dashed line vs. solid line) but not FFRs to the pseudo-CS+ (Fig. 4e, f, dashed line vs. solid line). For FFRs to the ipsilateral CS+ image (Fig. 4c) and FFRs to the contralateral CS+ image (Fig. 4d), 2 (separation type)  $\times$  4 (SNR) repeated-measures ANOVAs show similar results: spatial separation significantly enhanced LA FFRs (both  $F_{(1,11)} > 81.6$ ,  $p < 0.01$ ) without any significant interaction with SNR (both  $F_{(3,33)} < 2.5$ ,  $p > 0.05$ ). However, for FFRs to the ipsilateral pseudo-CS+ image (Fig. 4e) and FFRs to the contralateral pseudo-CS+ image (Fig. 4f), 2 (separation type)  $\times$  4 (SNR) repeated-measures ANOVAs indicate that neither the interaction between separation type and SNR (both  $F_{(3,33)} < 2$ ,  $p > 0.05$ ) nor the main effect of separation type (both  $F_{(1,11)} < 3.8$ ,  $p > 0.05$ ) was significant. Thus, perceived spatial separation between the CS and the noise masker could enhance LA responses to the emotionally salient CS with the stimulus specificity.

## DISCUSSION

### Fear conditioning elicits stimulus-specific plasticity in LA

Consistent with previous reports that auditory fear conditioning enhances CS-elicited LA responses (Maren et al., 1991; Quirk et al., 1995; Collins and Paré, 2000; Maren, 2000), the results of this study show that when a neutral tone-complex became fear conditioned, both LA FFRs to this CS+ increased and the latency of field-potential responses to this CS+ from the contralateral side decreased. However, the enhancement of LA FFRs did not occur if the tone-complex was manipulated with the conditioning-control procedure (the pseudo-CS+). Statistical analyses also confirm that LA FFRs to the CS+ were significantly larger than those to the pseudo-CS+ after the fear conditioning/conditioning-control manipulation. Thus, the conditioning-induced enhancement of LA FFRs to the CS+ is stimulus specific, supporting the view that the conditioned plasticity results from a process of CS–US associative learning with a stimulus specificity (Rogan et al., 1997; Collins and Paré, 2000; Goosens et al., 2003).

Also, since the FFRs are sustained potentials based on phase-locked firing of neuron populations to periodical sound waveforms, the results support the view that the LA is the core structure of conditioned plasticity represented by an increase not only in firing rate but also in the synchronization of neural activity (Fanselow and LeDoux, 1999; Blair et al., 2001; Maren and Quirk, 2004).

### Perceived spatial separation enhances LA FFRs to the fear-conditioned stimulus

When the two loudspeakers are spatially symmetrical to the listener, the precedence-effect-induced perceived spatial separation between the target stimulus and the masker does not substantially affect either the signal-to-masker ratio at the ear or the compactness/diffusiveness of the stimulus images (Li et al., 2004). In other words, changes in the perceived spatial relationship between the target and the masker do not affect the impact of bottom-up sensory inputs.

Previous studies have confirmed that the perceived spatial separation facilitates selective attention to the target stimulus in both humans (e.g., Freyman et al., 1999; Li et al., 2004) and rats (Du et al., 2009b, 2010, 2011b). In this study, before the tone-complex became fear conditioned, a shift from the condition of perceptual co-location to the condition of perceptual separation enhanced LA FFRs to the tone-complex merely at low SNRs (–8 and –4 dB) only when the tone-complex image was ipsilateral to the recording side. The results are consistent with previous reports that FFRs in both the LA and the IC of rats exhibit a feature of ipsilateral-input dominance which may result from the greater contribution of “EE” neurons (neurons are excited by stimuli at either ear) than other binaural neurons in the IC in forming binaural FFRs (Du et al., 2009a,c).

More importantly, following the tone-complex became fear conditioned, the perceptual separation generally enhanced LA FFRs to the conditioned tone-complex at each of the SNRs, regardless of whether the tone-complex image was ipsilateral or contralateral to the recording side. Also, the separation-induced enhancement after fear conditioning was stimulus-specific: it did not occur for LA FFRs to the pseudo-CS+. Thus, this study provided evidence that in rats the formation of selective attention to an ecologically-relevant (fear-conditioned) acoustic stimulus is correlated with the enhancement of LA responses to the stimulus. The results particularly agree with the behavioral report that the amygdala mediates fear-related attention toward the most salient signal, such as a threat, under stressful circumstances (Meck and MacDonald, 2007). Moreover, the results of this study support the results of human studies showing attentional modulation of amygdala responses to emotionally salient stimuli (Pessoa et al., 2002; Bishop et al., 2007; Straube et al., 2007; Mothes-Lasch et al., 2011). However, it should be noted that recent studies in humans have also shown that amygdala responses to an emotional stimulus contain both the automatic and attentionally controlled components (Luo et al., 2010; Pourtois et al., 2010; Shafer et al., 2012). More specifically, using the high temporal resolution method of either magnetoencephalography (MEG) (Luo et al., 2010) or intracranial local field potential recordings (Pourtois et al., 2010), it has been revealed that the early amygdala response to emotional information is independent of attentional modulation but the later amygdala response exhibits remarkable attentional modulation. Thus, the discrepancies of the views about

the automaticity/non-automaticity of amygdala responses to emotional stimuli can be reconciled (see Brosch and Wieser, 2011).

It would be of interest to know the neural pathways mediating attentional modulation of LA activity, because these pathways may be related to the facilitation of selective attention to the target when the target is perceived as spatially separated from the masker due to the precedence effect. It has been known that the posterior parietal cortex (PPC) mediates spatial attention shift/orienting in humans (e.g., Yantis et al., 2002) and directed spatial attention in rats (e.g., Reep and Corwin, 2009). Also, one recent study has shown that pharmacological blockade of the PPC eliminates the enhancing effect of perceptual spatial separation on PPI elicited by the fear-conditioned target sound in rats (Du et al., 2011b). The PPC has reciprocal neural connections with both the auditory cortex and the medial prefrontal cortex (Reep et al., 1994), and the two brain structures send axonal projections to the amygdala (Romanski and LeDoux, 1993; McDonald et al., 1996). Thus, the enhancement of CS-evoked LA responses under the condition of perceptual separation may be attributed to the functional connections between the PPC and the LA.

## CONCLUSIONS

For the first time, this study demonstrates attentional modulation of LA responses to the emotionally salient stimuli in awake rats under noise-masking conditions where the bottom-up sensory-input impacts were kept constant. Although amygdala activations to emotionally salient signals may contain automatic components, a facilitation of allocating attentional resources to the emotionally salient stimulus can further enhance the neural representation of the stimulus in the LA, particularly under masking circumstances. Thus, the interaction between emotion and attention mediated by reciprocating neural connections between amygdala and some cortical regions, such as the PPC, may contribute to more efficient and adaptive actions to the ecologically important events in challenging (e.g., noisy, reverberant) environments.

*Acknowledgements—This work was supported by the National Natural Science Foundation of China (30950030), the “973” National Basic Research Program of China (2009CB320901), the Chinese Ministry of Education (20090001110050), and “985” Grants from Peking University.*

## REFERENCES

- Anderson AK, Christoff K, Panitz D, De Rosa E, Gabrieli JD (2003) Neural correlates of the automatic processing of threat facial signals. *J Neurosci* 23:5627–5633.
- Bishop SJ, Jenkins R, Lawrence AD (2007) Neural processing of fearful faces: effects of anxiety are gated by perceptual capacity limitations. *Cereb Cortex* 17:1595–1603.
- Blair HT, Schafe GE, Bauer EP, Rodrigues SM, LeDoux JE (2001) Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. *Learn Mem* 8:229–242.
- Brosch T, Wieser MJ (2011) The (non)automaticity of amygdala responses to threat: on the issue of fast signals and slow measures. *J Neurosci* 31:14451–14452.
- Brungart DS, Simpson BD, Freyman RL (2005) Precedence-based speech segregation in a virtual auditory environment. *J Acoust Soc Am* 118:3241–3251.
- Collins DR, Paré D (2000) Differential fear conditioning induces reciprocal changes in the sensory responses of lateral amygdala neurons to the CS(+) and CS(–). *Learn Mem* 7:97–103.
- Du Y, Huang Q, Wu XH, Galbraith GC, Li L (2009a) Binaural unmasking of frequency-following responses in rat amygdala. *J Neurophysiol* 101:1647–1659.
- Du Y, Li JY, Wu XH, Li L (2009b) Precedence-effect-induced enhancement of prepulse inhibition in socially reared but not isolation-reared rats. *Cogn Affect Behav Neurosci* 9:44–58.
- Du Y, Ma TF, Wang Q, Wu XH, Li L (2009c) Two crossed axonal projections contribute to binaural unmasking of frequency-following responses in rat inferior colliculus. *Eur J Neurosci* 30:1779–1789.
- Du Y, Wu X, Li L (2010) Emotional learning enhances stimulus-specific top-down modulation of sensorimotor gating in socially reared rats but not isolation-reared rats. *Behav Brain Res* 206:192–201.
- Du Y, Kong LZ, Wang Q, Wu XH, Li L (2011a) Auditory frequency-following responses: a neurophysiological measure for studying the “cocktail-party problem”. *Neurosci Biobehav Rev* 35:2046–2057.
- Du Y, Wu XH, Li L (2011b) Differentially organized top-down modulation of prepulse inhibition of startle. *J Neurosci* 31:13644–13653.
- Fanselow MS, LeDoux JE (1999) Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron* 23:229–232.
- Foss JA, Ison JR, Torre Jr JP, Wansack S (1989) The acoustic startle response and disruption of aiming: I. Effect of stimulus repetition, intensity, and intensity changes. *Hum Factors* 31:307–318.
- Freyman RL, Clifton RK, Litovsky RY (1991) Dynamic processes in the precedence effect. *J Acoust Soc Am* 90:874–884.
- Freyman RL, Helfer KS, McCall DD, Clifton RK (1999) The role of perceived spatial separation in the unmasking of speech. *J Acoust Soc Am* 106:3578–3588.
- Goosens KA, Hobin JA, Maren S (2003) Auditory-evoked spike firing in the lateral amygdala and Pavlovian fear conditioning: mnemonic code or fear bias? *Neuron* 40:1013–1022.
- Hoeffding V, Harrison JM (1979) Auditory discrimination: role of time and intensity in the precedence effect. *J Exp Anal Behav* 32:157–166.
- Hoffman HS, Searle JL (1965) Acoustic variables in the modification of startle reaction in the rat. *J Comp Physiol Psychol* 60:53–58.
- Hoffman HS, Overman W (1971) Performance disruption by startle-eliciting acoustic stimuli. *Psychonom Sci* 24:233–235.
- Hoffman HS, Ison JR (1980) Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol Rev* 87:175–189.
- Huang J, Yang ZG, Ping JL, Liu X, Wu XH, Li L (2007) The influence of the perceptual or fear learning on rats’ prepulse inhibition induced by changes in the correlation between two spatially separated noise sounds. *Hear Res* 223:1–10.
- Huang Y, Huang Q, Chen X, Qu TS, Wu XH, Li L (2008) Perceptual integration between target speech and target-speech reflection reduces masking for target-speech recognition in younger adults and older adults. *Hear Res* 244:51–65.
- Huang Y, Huang Q, Chen X, Wu XH, Li L (2009) Transient auditory storage of acoustic details is associated with release of speech from informational masking in reverberant conditions. *J Exp Psychol Hum Percept Perform* 35:1618–1628.
- Huang Y, Li JY, Zou XF, Qu TS, Wu XH, Mao LH, Wu YH, Li L (2011) Perceptual fusion tendency of speech sounds. *J Cog Neurosci* 23:1003–1014.



- Kelly JB (1974) Localization of paired sound sources in the rat: small time difference. *J Acoust Soc Am* 55:1277–1284.
- Kidd Jr G, Mason CR, Brughera A, Hartmann WM (2005) The role of reverberation in release from masking due to spatial separation of sources for speech identification. *Acta Acust United Acust* 91:526–536.
- Koch M (1999) The neurobiology of startle. *Prog Neurobiol* 59:107–128.
- Koehnke J, Besing JM (1996) A procedure for testing speech intelligibility in a virtual listening environment. *Ear Hear* 17:211–217.
- LeDoux JE, Farb CR, Ruggiero DA (1990) Topographic organization of neurons in the acoustic thalamus that project to the amygdala. *J Neurosci* 10:1043–1054.
- Li L, Daneman M, Qi JG, Schneider BA (2004) Does the information content of an irrelevant source differentially affect speech recognition in younger and older adults? *J Exp Psychol Hum Percept Perform* 30:1077–1091.
- Li L, Qi JG, He Y, Alain C, Schneider B (2005) Attribute capture in the precedence effect for long-duration noise sounds. *Hear Res* 202:235–247.
- Li L, Du Y, Li NX, Wu XH, Wu YH (2009) Top-down modulation of prepulse inhibition the startle reflex in humans and rats. *Neurosci Biobehav Rev* 33:1157–1167.
- Li NX, Ping JL, Wu RB, Wang C, Wu XH, Li L (2008) Auditory fear conditioning modulates prepulse inhibition in socially-reared rats and isolation-reared rats. *Behav Neurosci* 122:107–118.
- Litovsky RY, Colburn HS, Yost WA, Guzman SJ (1999) The precedence effect. *J Acoust Soc Am* 106:1633–1654.
- Luo Q, Holroyd T, Majestic C, Cheng X, Schechter J, Blair RJ (2010) Emotional automaticity is a matter of timing. *J Neurosci* 30:5825–5829.
- Maren S (2000) Auditory fear conditioning increases CS-elicited spike firing in lateral amygdala neurons even after extensive overtraining. *Eur J Neurosci* 12:4047–4054.
- Maren S (2011) Seeking a spotless mind: extinction, deconsolidation, and erasure of fear memory. *Neuron* 70:830–845.
- Maren S, Poremba A, Gabriel M (1991) Basolateral amygdaloid multiunit neuronal correlates of discriminative avoidance-learning in rabbits. *Brain Res* 549:311–316.
- Maren S, Quirk GJ (2004) Neuronal signalling of fear memory. *Nat Rev Neurosci* 5:844–852.
- McDonald AJ, Mascagni F, Guo L (1996) Projections of the medial and lateral prefrontal cortices to the amygdala: a *Phaseolus vulgaris* leucoagglutinin study in the rat. *Neuroscience* 71:55–75.
- Meck WH, MacDonald CJ (2007) Amygdala inactivation reverses fear's ability to impair divided attention and make time stand still. *Behav Neurosci* 121:707–720.
- Mothes-Lasch M, Mentzel HJ, Miltner WHR, Straube T (2011) Visual attention modulates brain activation to angry voices. *J Neurosci* 31:9594–9598.
- Pasley BN, Mayes LC, Schultz RT (2004) Subcortical discrimination of unperceived objects during binocular rivalry. *Neuron* 42:163–172.
- Pessoa L, McKenna M, Gutierrez E, Ungerleider LG (2002) Neural processing of emotional faces requires attention. *Proc Natl Acad Sci USA* 99:11458–11463.
- Phelps EA, LeDoux JE (2005) Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron* 48:175–187.
- Ping JL, Li NX, Galbraith GC, Wu XH, Li L (2008) Auditory frequency-following responses in rat ipsilateral inferior colliculus. *NeuroReport* 19:1377–1380.
- Pourtois G, Spinelli L, Seeck M, Vuilleumier P (2010) Temporal precedence of emotion over attention modulations in the lateral amygdala: intracranial ERP evidence from a patient with temporal lobe epilepsy. *Cogn Affect Behav Neurosci* 10:83–93.
- Quirk GJ, Reppas CB, LeDoux JE (1995) Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. *Neuron* 15:1029–1039.
- Rakerd B, Aaronson NL, Hartmann WM (2006) Release from speech-on-speech masking by adding a delayed masker at a different location. *J Acoust Soc Am* 119:1597–1605.
- Reep RL, Chandler HC, King V, Corwin JV (1994) Rat posterior parietal cortex-topography of corticocortical and thalamic connections. *Exp Brain Res* 100:67–84.
- Reep RL, Corwin JV (2009) Posterior parietal cortex as part of a neural network for directed attention in rats. *Neurobiol Learn Mem* 91:104–113.
- Rogan MT, Staubli UV, LeDoux JE (1997) Fear conditioning induces associative long-term potentiation in the amygdala. *Nature* 390:604–607.
- Romanski LM, LeDoux JE (1993) Information cascade from primary auditory cortex to the amygdala-corticocortical and corticoamygdaloid projections of temporal cortex in the rat. *Cereb Cortex* 3:515–532.
- Shafer AT, Matveychuk D, Penney T, O'Hare AJ, Stokes J, Dolcos F (2012) Processing of emotional distraction is both automatic and modulated by attention: evidence from an event-related fMRI investigation. *J Cog Neurosci* 24:1233–1252.
- Spitzer MW, Takahashi TT (2006) Sound localization by barn owls in a simulated echoic environment. *J Neurophysiol* 95:3571–3584.
- Straube T, Weiss T, Mentzel HJ, Miltner WHR (2007) Time course of amygdala activation during aversive conditioning depends on attention. *NeuroImage* 34:462–469.
- Vuilleumier P, Armony JL, Driver J, Dolan RJ (2001) Effects of attention and emotion on face processing in the human brain: an event-related fMRI study. *Neuron* 30:829–841.
- Wallach H, Newman EB, Rosenzweig MR (1949) The precedence effect in sound localization. *Am J Psychol* 62:315–336.
- Whalen PJ, Rauch SL, Etcoff NL, Mclnerney SC, Lee MB, Jenike MA (1998) Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. *J Neurosci* 18:411–418.
- Worden FG, Marsh JT (1968) Frequency-following (microphonic-like) neural responses evoked by sound. *Electroencephalograp Clin Neurophysiol* 25:42–52.
- Wu XH, Wang C, Chen J, Qu HW, Li WR, Wu YH, Schneider BA, Li L (2005) The effect of perceived spatial separation on informational masking of Chinese speech. *Hear Res* 199:1–10.
- Yantis S, Schwarzbach J, Serences JT, Carlson RL, Steinmetz MA, Pekar JJ, Courtney SM (2002) Transient neural activity in human parietal cortex during spatial attention shifts. *Nat Neurosci* 5:995–1002.
- Yeomans JS, Li L, Scott BW, Frankland PW (2002) Tactile, acoustic and vestibular systems sum to elicit the startle reflex. *Neurosci Biobehav Rev* 26:1–11.
- Zheng JW, Wu XH, Li L (2008) Metabotropic glutamate receptors subtype 5 are necessary for the enhancement of auditory evoked potentials in the lateral nucleus of the amygdala by tetanic stimulation of the auditory thalamus. *Neuroscience* 152:254–264.
- Zou D, Huang J, Wu XH, Li L (2007) Metabotropic glutamate subtype 5 receptors modulate fear-conditioning induced enhancement of prepulse inhibition in rats. *Neuropharmacology* 52:476–486.
- Zurek PM (1980) The precedence effect and its possible role in the avoidance of interaural ambiguities. *J Acoust Soc Am* 67:953–964.
- Zurek PM, Freyman RL, Balakrishnan U (2004) Auditory target detection in reverberation. *J Acoust Soc Am* 115:1609–1620.