Behavioural Brain Research 269 (2014) 87–94

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Perceived location specificity in perceptual separation-induced but not fear conditioning-induced enhancement of prepulse inhibition in rats

Ming Lei[a], Lu Luo[a], Tianshu Qu[b], Hongxiao Jia[c], Liang Li[a,*]

[a] Department of Psychology, McGovern Institute for Brain Research, Key Laboratory on Machine Perception (Ministry of Education), Peking University, Beijing 100871, China
[b] Department of Machine Intelligence, Speech and Hearing Research Center, Peking University, Beijing 100871, China
[c] Beijing An Ding Hospital, Capital Medical University, Beijing 100088, China

HIGHLIGHTS

- Fear conditioning enhances PPI without exhibiting a spatial location specificity.
- Perceptual spatial separation between the conditioned prepulse and the noise masker enhances PPI with a spatial location specificity.
- Both types of PPI enhancements can be abolished by extinction learning, which depends on metabotropic glutamate receptors subtype 5.

ARTICLE INFO

Article history:
Received 30 March 2014
Received in revised form 14 April 2014
Accepted 16 April 2014
Available online 26 April 2014

Keywords:
Prepulse inhibition
Fear conditioning
Precedence effect
Perceptual spatial separation
Perceived location specificity
Metabotropic glutamate receptor

ABSTRACT

Prepulse inhibition (PPI) is the suppression of the startle reflex when the startling stimulus is shortly preceded by a non-startling stimulus (the prepulse). Previous studies have shown that both fear conditioning of a prepulse and precedence-effect-induced perceptual separation between the conditioned prepulse and a noise masker facilitate selective attention to the prepulse and consequently enhance PPI with a remarkable prepulse-feature specificity. This study investigated whether the two types of attentional enhancements of PPI in rats also exhibit a prepulse-location specificity. The results showed that when a prepulse was delivered by each of the two spatially separated loudspeakers, fear conditioning of the prepulse at a particularly perceived location (left or right to the tested rat) enhanced PPI without exhibiting any perceived-location specificity. However, when a noise masker was presented, the precedence-effect-induced perceptual separation between the conditioned prepulse and the noise masker further enhanced PPI when the prepulse was perceived as coming from the location that was conditioned but not the location without being conditioned. Moreover, both conditioning-induced and perceptual separation-induced PPI enhancements were eliminated by extinction learning, whose effect could be blocked by systemic injection of the selective antagonist of metabotropic glutamate receptor subtype 5 (mGluR5), 2-methyl-6-(phenylethynyl)-pyridine (MPEP). Thus, fear conditioning of a prepulse perceived at a particular location not only facilitates selective attention to the conditioned prepulse but also induces a learning-based spatial gating effect on the spatial unmasking of the conditioned prepulse, leading to that the perceptual separation-induced PPI enhancement becomes perceived-location specific.

1. Introduction

The startle reflex is the whole-body reflexive response to sudden and intense sensory stimuli [1] and disrupts cognitive/behavioral performances [2,3]. Prepulse inhibition (PPI) is the suppression of the startle reflex when a weaker sensory stimulus (the prepulse) shortly precedes the startling stimulus [2,4]. The "protection-of-processing" theory [5] suggests that receiving a prepulse simultaneously triggers the information processing for the prepulse and the gating mechanism dampening the disrupting effect of the startling input. Since PPI can reduce the behavioral responses to disruptive stimuli by regulating the motor/premotor system, it has been widely used as an operational measure of sensorimotor gating.

* Corresponding author. Tel.: +86 10 62756804; fax: +86 10 62761081.
E-mail addresses: liangli@pku.edu.cn, liangli2@hotmail.com (L. Li).

http://dx.doi.org/10.1016/j.bbr.2014.04.030
0166-4328/

The primary neural circuitry mediating PPI is located in the brainstem ([6,7], for review see [8]), indicating that PPI is mainly an automatic process at pre-attentive stages. However, PPI can be modulated by attention to the prepulse in both humans and rats [9–20]. More specifically, in rats when a prepulse becomes fear conditioned, it draws more attention and elicits larger PPI [13,16–20]. The conditioning-induced PPI enhancement in rats also exhibits a marked prepulse-feature specificity [13,17]. Moreover, when a noise masker is presented, introducing a precedence-effect-based perceptual separation between the noise masker and the conditioned prepulse further enhances PPI with the prepulse-feature specificity [13,16,17] (see below).

What is the precedence effect? In a reverberant environment, listeners have the ability to perceptually integrate the direct sound wave and the reflections of a sound source: attributes of the delayed and correlated reflection are perceptually captured by the direct wave [21], leading to a single fused image whose perceived point of origin is around the location of the leading source (i.e., the "precedence effect" [22,23]). In humans, when both the target sound (e.g., a speech) and the masker (a noise or speech) are presented by each of the two spatially separated loudspeakers with an inter-loudspeaker delay of 3 ms, recognizing the target speech under the condition of perceived target-masker spatial separation (when the leading loudspeaker was different between target and masker) is significantly better than that under the condition of perceived co-location (when the leading loudspeaker was the same for both target and masker). Note that shifts between the perceived separation condition and the perceived co-location condition do not substantially change the signal-to-masker ratio in sound pressure level and the sound-image compactness/diffusiveness [21,24]. The reduction of masking is caused by higher-order processes including the improvement of selective attention to the target.

In awake rats, when a fear-conditioned target sound and a noise masker are delivered by each of the two spatially separated loudspeakers with the inter-loudspeaker delay of 1 ms, the precedence-effect-induced perceptual separation between the target sound and the noise masker facilitates the rat's attention to the target and enhances auditory responses of the amygdala to the target sound [25]. Moreover, when the precedence-effect-induced perceived spatial separation is introduced between a conditioned prepulse and a noise masker, the conditioned prepulse-induced PPI is further enhanced due to the facilitation of the rat's selective attention to the conditioned prepulse [13,16,17]. The perceptual separation-induced PPI enhancement also exhibits the prepulse-feature specificity. However, it is still unclear whether a spatial location specificity also occurs in the fear conditioning-induced and/or the perceptual separation-induced attentional enhancements of PPI. This issue was investigated in this study.

In people with schizophrenia, there is evidence showing that impairment of the attentional modulation of PPI, but not impairment of the baseline PPI, is significantly correlated with the symptom severity of this disorder ([10,11,14,15], for a review see [12]). In rats with social isolation rearing, both fear conditioning-induced PPI enhancement [13,16,19,20] and perceptual separation-induced PPI enhancement [13,16] completely disappear. Thus, studies of both the fear conditioning-induced and the perceptual separation-induced enhancements of PPI in rats are useful for establishing new animal models of schizophrenia. Since dysfunctions of spatial selective attention have also been reported in people of schizophrenia [26–28], investigation of whether the PPI enhancements exhibit a location specificity is undoubtedly critical to improve the modeling studies.

Furthermore, previous studies have shown that both the conditioning-induced and perceptual-separation-induced PPI enhancements can be abolished by extinction learning [13,16,17]. The metabotropic glutamate receptor subtype 5 (mGluR5) is essential for both the formation of the conditioning-induced PPI enhancement [19,20] and the extinction of fear conditioning [29,30]. However, it is not clear whether mGluR5 plays a role in mediating the extinction of the PPI enhancements. Using the selective antagonist of mGluR5, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), this study also examined whether the extinction of fear-conditioning-induced and/or perceptual separation-induced PPI enhancements are mGluR5 dependent.

2. Materials and methods

2.1. Animals

Forty-eight adult male Sprague-Dawley rats (280–350 g, the Vital-River Experimental Animals Technology Ltd., Beijing, China) were used in this study. These rats were assigned randomly into four groups (12 rats in each group) with various combinations of conditioning manipulations and injection agents: (1) fear conditioning/saline, (2) fear conditioning/MPEP, (3) conditioning-control/saline, and (4) conditioning-control/MPEP.

All the rats were kept in a room with the temperature of $24 \pm 2^{\circ}$C and a 12 h light/dark cycle, with food and water available at libitum. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The rats 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 approved by the Society for Neuroscience (2006). The procedures of this study were approved by the Committee for Protecting Human and Animal Subjects, the Department of Psychology at Peking University.

2.2. Stimuli and apparatus

The apparatus for PPI testing have been described in detail elsewhere [20]. Briefly, the rat's whole-body startle reflex, which was induced by an intense 10-ms broadband noise burst (0–10 kHz, 100 dB SPL) delivered by a loudspeaker above the rat's head, was measured by a custom-made electrical scale (the National Key Laboratory on Machine Perception, Peking University) in a soundproof chamber. Beginning with the onset of the startling stimulus, electrical voltages were collected and sampled at a frequency of 16 kHz for 500 ms. For each trial, the peak-to-peak amplitude of the startle response was digitized and measured [20].

The prepulse stimulus was delivered by each of the two spatially separated (i.e., left and right) loudspeakers in the frontal field with a 100° separation angle and 52 cm away from the rat's head position (Fig. 1a). The prepulse, which started 100 ms before the startling sound, was a 50-ms three-harmonic tone complex (2.3, 4.6 and 6.9 kHz). It was digitally generated by MATLAB software (the MathWorks Inc., Natick, MA, USA) and converted by a custom-developed sound-delivery system (the National Key Laboratory on Machine Perception, Peking University). The single-source sound level of the prepulse for each of the two horizontal loudspeakers was fixed at 60 dB SPL. Calibration of sound intensity was carried out with a Larson Davis Audiometer Calibration and Electroacoustic Testing System (AUDit and System 824, Larson Davis, Depew, NY, USA) whose microphone was placed at the central location of the rat's head when the rat was absent.

2.3. Testing procedures

2.3.1. Adaptation

Each rat went through an eight-day testing procedure. For the first three successive days, the rat was placed in a restraining cage, whose dimensions matched the size of the rat, and the rat could not re-orient its body position. For 30 min, the rat was exposed to the

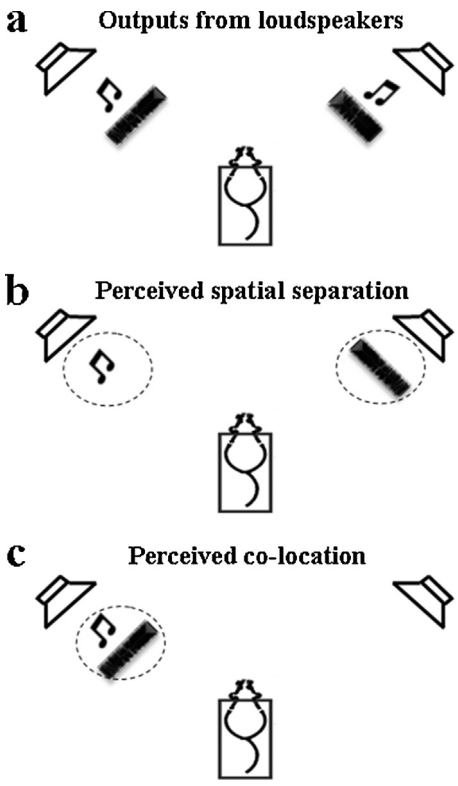

Fig. 1. Diagrams showing the physical (Panel a) and perceived (Panels b and c) spatial relationship between the prepulse (the music note) and the noise masker (noise waveform). (Panel a) Both the prepulse and the noise masker were delivered by each of the two horizontally separated loudspeakers. (Panel b) The onset of the prepulse delivered by the left loudspeaker led that from the right loudspeaker by 1 ms, and the image of the prepulse (music note in the circle) was perceived as coming from the left loudspeaker; the onset of the masker from the right loudspeaker led that from the left loudspeaker by 1 ms, and the image of the masker (noise waveform in circle) was perceived as coming from the right loudspeaker. Thus, the prepulse and the noise masker were perceived spatially separated. (Panel c) Both the onset of the prepulse and the onset of the masker presented from the left loudspeaker led those from the right loudspeaker, and the image of the prepulse (music note in circle) and that of the masker (noise waveform in circle) were perceived spatially co-located.

broadband noise (0–10 kHz, 60 dB SPL), which was continuously presented by each of the two horizontal loudspeakers. This procedure aimed to adapt the rat to the restraining cage and testing chamber.

2.3.2. PPI baseline

On the fourth day, the baseline PPI before the conditioning/conditioning-control manipulations was measured (procedure stage BC). The rat was placed in the restraining cage and received 10 presentations of the startling sound without prepulse presentation for 5 min. Then 4 testing blocks were conducted. In each of the blocks, 5 trials contained the startling sound alone delivered by the top loudspeaker, and 10 trials contained the prepulse 100 ms preceding the startling noise (50 ms between the prepulse offset and the startling-sound onset). The prepulse was presented from each of the two horizontal loudspeakers with the inter-loudspeaker onset delay being either +1 ms (left leading) or −1 ms (right leading). Due to the precedence effect [21–23], a single perceptually fused image of the prepulse would be perceived at the location of the left loudspeaker in 2 of the 4 blocks (when the left loudspeaker led) and at the location of the right loudspeaker in the other 2 blocks (when the right loudspeaker led). In addition to the prepulse, a background wideband noise (0–10 kHz, 60 dB SPL) was continuously delivered from each of the two horizontal loudspeakers as the masker. The inter-loudspeaker onset delay for the masker was also either +1 or −1 ms, leading to a perceptually fused continuous noise-masker image either at the left loudspeaker in 2 blocks or at the right loudspeaker in the other 2 blocks. Thus, there were 4 (2×2) combinations of perceived locations between the prepulse and masker images across the 4 blocks. Two types of perceived spatial relations between the prepulse and masker were created: perceptual separation (when prepulse and masker had different leading loudspeakers, Fig. 1b) and perceptual co-location (when prepulse and masker shared the same leading loudspeaker, Fig. 1c). Trials in each block were presented randomly with the inter-trial interval about 30 s (vary between 25 and 30 s). The order of presenting the four blocks was counterbalanced among rats in each group.

2.3.3. Fear conditioning/conditioning-control manipulations

On the fifth day, all the rats underwent the fear-conditioning manipulation or the conditioning-control manipulation. In each of the 2 conditioning groups, half of the rats were left-location conditioned when the conditioned stimulus (CS+) was the prepulse pair with the left loudspeaker leading, and the other half were right-location conditioned. In each of the 2 conditioning-control groups, half of the rats were left-location control-conditioned when the control-conditioned stimulus (CS−) was the prepulse pair with the left loudspeaker leading, and the other half were right-location control-conditioned. Note that for each rat, only one of the perceived prepulse locations (either the left or right loudspeaker) received the fear-conditioning or conditioning-control manipulation. Using a Grass S-88 stimulator (Grass, Quincy, MA, USA) [20], the unconditioned stimulus (US) was a 6-mA rectangular-pulse footshock with a duration of 3 ms. For the conditioning groups, 20 temporally synchronized (paired) combinations of the footshock (i.e., US) and the prepulse (i.e., CS+) were presented every 30 s (US started 3 ms before CS+ ending, and co-terminated with CS+). For the conditioning-control groups, 20 temporally random (unpaired) combinations of the US and CS− were presented every 30 s.

2.3.4. PPI after fear conditioning/conditioning-control manipulations

On the sixth day (24 h after fear-conditioning or conditioning-control manipulations), PPI after conditioning (procedure stage AC) was measured with the same procedure as on the fourth day. Note that after the conditioning or conditioning-control manipulations, the prepulse was then perceived either at the manipulation-associated location (with conditioning or conditioning-control manipulation) or at the location without any manipulations.

2.3.5. Drug injection and extinction learning

On the seventh day, all rats underwent systemic injection of either MPEP ($C_{14}H_{11}N{\cdot}HCl$, Sigma–Aldrich Corporate, St Louis, MO, USA) or saline and fear extinction learning. MPEP solution was freshly prepared with 0.9% saline and administered 30 min before extinction learning in the 2 MPEP-injection groups (5 mg/kg, i.p.). For the 2 saline groups, only saline (1 ml, i.p.) was administered 30 min before extinction learning.

Thirty minutes after injection, the extinction-learning manipulation was conducted without delivering footshock (US). The prepulse from the perceived location with conditioning or conditioning control was presented 60 times with the inter-stimulus interval of 30 s without presenting the noise masker. For each rat, the 60 prepulse presentations were evenly divided into 3 extinction-learning sessions with the intersession interval of 10 min.

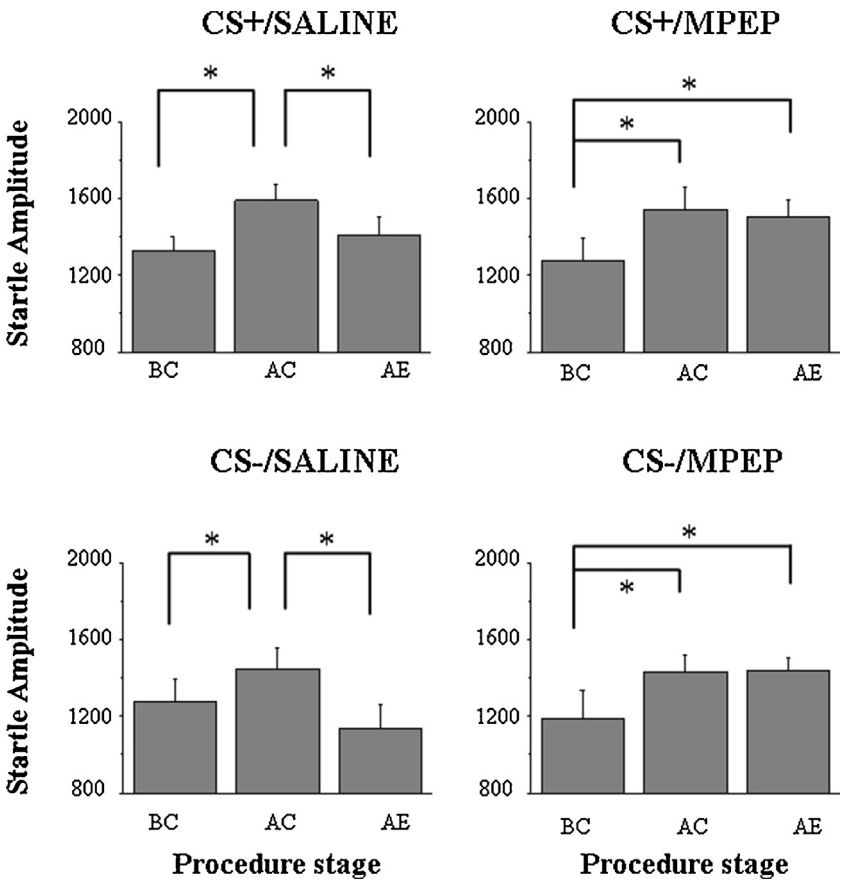

Fig. 2. Amplitudes of startling responses to the startling stimulus alone in each of the four rat groups. CS+, the prepulse that was fear conditioned; CS−, the prepulse that was control-conditioned; SALINE, saline injection; MPEP, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) injection; BC, before conditioning; AC, after conditioning; AE, after extinction. Error bars represent the standard error of the mean. $^{*}p < 0.05$.

2.3.6. PPI after extinction learning

On the eighth day, PPI after extinction learning was measured (procedure stage AE) with the same procedure as on the fourth day.

2.4. Data analyses

The PPI value was calculated with the following generally used formula:

$$\text{PPI value} = \frac{(\text{startle amplitude to startling sound alone} - \text{startle amplitude to startling sound preceded by prepulse})}{(\text{startle amplitude to startling sound alone})}$$

In each group, half of the rats received the fear conditioning or conditioning-control manipulation with the prepulse image perceived at the left-loudspeaker location and the other half at the right-loudspeaker location. Since the results showed that there was no significant difference between the two subgroups, PPI values were averaged within each group. Analyses of variance (ANOVAs) were conducted followed by Bonferroni pairwise comparisons and paired *t*-tests, using SPSS 15.0 software. The null-hypothesis rejection level was set at 0.05.

3. Results

3.1. Responses to the startling sound alone

Fig. 2 shows the group-mean startle amplitudes to the startling stimulus alone in each of the four rat groups (CS+/saline, CS+/MPEP, CS−/saline, CS−/MPEP) before and after conditioning/conditioning-control manipulations and after the extinction-learning manipulation. Generally the startle amplitude was increased by either the fear-conditioning manipulation (top panels) or the conditioning-control manipulation (bottom panels), and was decreased by the extinction manipulation if saline (left panels), but not MPEP (right panels), was injected before extinction learning.

A 3 (procedure stage: BC, AC, AE) by 2 (manipulation: conditioning, conditioning-control) by 2 (drug: saline, MPEP) three-way mixed ANOVA showed that the main effect of procedure stage was significant ($F_{(2,88)} = 10.78$, $p < 0.01$), the interaction between procedure stage and drug was significant ($F_{(2,88)} = 3.99$, $p < 0.05$), but the other main effects and interactions were not significant (all $p > 0.05$).

Post hoc tests showed that for the two saline-injection groups, significant differences in startle amplitude occurred between procedure stage BC and procedure AC ($p < 0.05$), between procedure stage AC and procedure stage AE ($p < 0.05$), but not between procedure stage BC and procedure AE ($p > 0.05$). For the two MPEP-injection groups, there were significant differences in startle amplitude between procedure stage BC and AC ($p < 0.05$), between procedure stage BC and AE ($p < 0.05$), but not between procedure stage AC and AE ($p > 0.05$).

These results indicate that (1) both the fear-conditioning manipulation and the conditioning-control manipulation significantly enhanced the startle amplitude, (2) extinction learning removed all the enhancing effects, and (3) the extinction learning effect was abolished by MPEP injection.

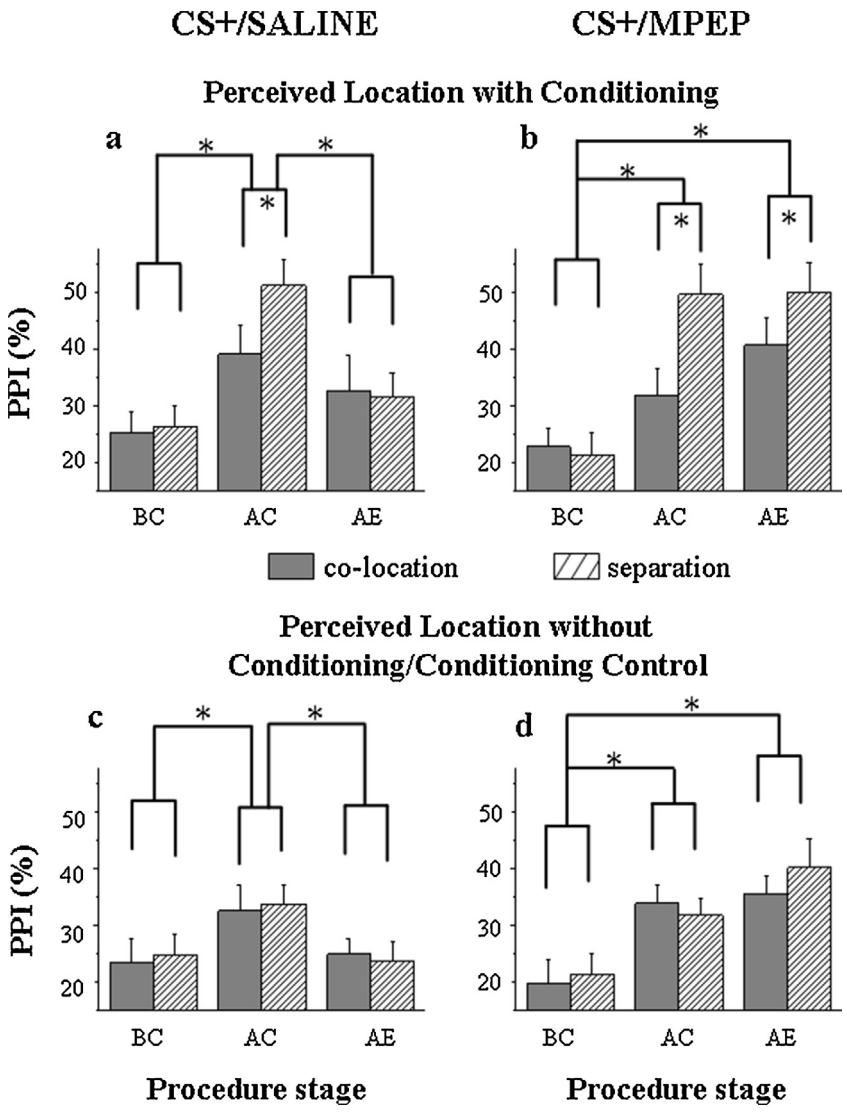

Fig. 3. Amplitudes of PPI in the two CS+ groups at different procedure stages. The top two panels present the PPI levels when the prepulse was perceived at the location with conditioning, and bottom two panels present the PPI levels when the prepulse was perceived from the perceived location without the conditioning or conditioning-control manipulation. Black bars represent the condition when the prepulse was perceptually co-located with the noise masker, while diagonal bars represent the condition when the prepulse was perceptually separated with the noise masker. $^{*}p < 0.05$.

3.2. Modulation of PPI in the two fear-conditioning groups

Two rat groups (CS+/saline, CS+/MPEP) received the fear-conditioning manipulation (CS+ was precisely paired with the footshock). Fig. 3 shows that following the conditioning manipulation (procedure stage AC), the PPI level in both the CS+/saline group (left panels) and the CS+/MPEP group (right panels) was markedly increased. However, the perceptual separation between the conditioned prepulse and the noise masker further enhanced PPI only when the prepulse was perceived as coming from the location with conditioning. Moreover, in rats with saline injection (left panels), but not in rats with MPEP injection (right panels), the PPI value reduced to the level as that before the conditioning manipulation (i.e., that at procedure stage BC) after the extinction learning manipulation (procedure stage AE).

3.2.1. The CS+/saline group

For the CS+/saline group (Fig. 3 left panels), a 2 (perceived prepulse location: with conditioning, without conditioning) by 3 (procedure stage: BC, AC, AE) by 2 (separation type: perceived separation, perceived co-location) within-subject repeated-measures ANOVA showed that the main effect of perceived prepulse location was significant ($F_{(1,11)} = 6.997$, $p < 0.05$), the main effect of procedure stage was significant ($F_{(2,22)} = 16.38$, $p < 0.01$), and the three-way interaction was significant ($F_{(2,22)} = 5.04$, $p < 0.05$).

A further separate 3 (procedure stage) by 2 (separation type) within-subject repeated-measures ANOVA showed that when the prepulse was perceived as coming from the location with conditioning (Fig. 3a), all the main effects and interaction were significant (all $F > 5.16$, $p < 0.05$). Post hoc tests showed that (1) at procedure stage BC, the PPI level was not affected by separation type ($p > 0.05$); (2) after fear conditioning (procedure stage AC), the PPI level was significantly enhanced ($p < 0.05$), and the effect of separation type became significant ($t_{(11)} = 2.73$, $p < 0.05$), confirming that PPI was significantly larger when the prepulse and noise masker were perceived spatially separated than perceived co-located; (3) at procedure stage AE (after extinction learning), all the PPI levels reduced to the one at procedure stage BC ($p > 0.05$), and the effect of separation type became not significant ($t_{(11)} = 0.21$, $p > 0.05$), showing the extinction-learning effect.

When the prepulse was perceived as coming from the location without conditioning (Fig. 3c), the main effect of procedure stage

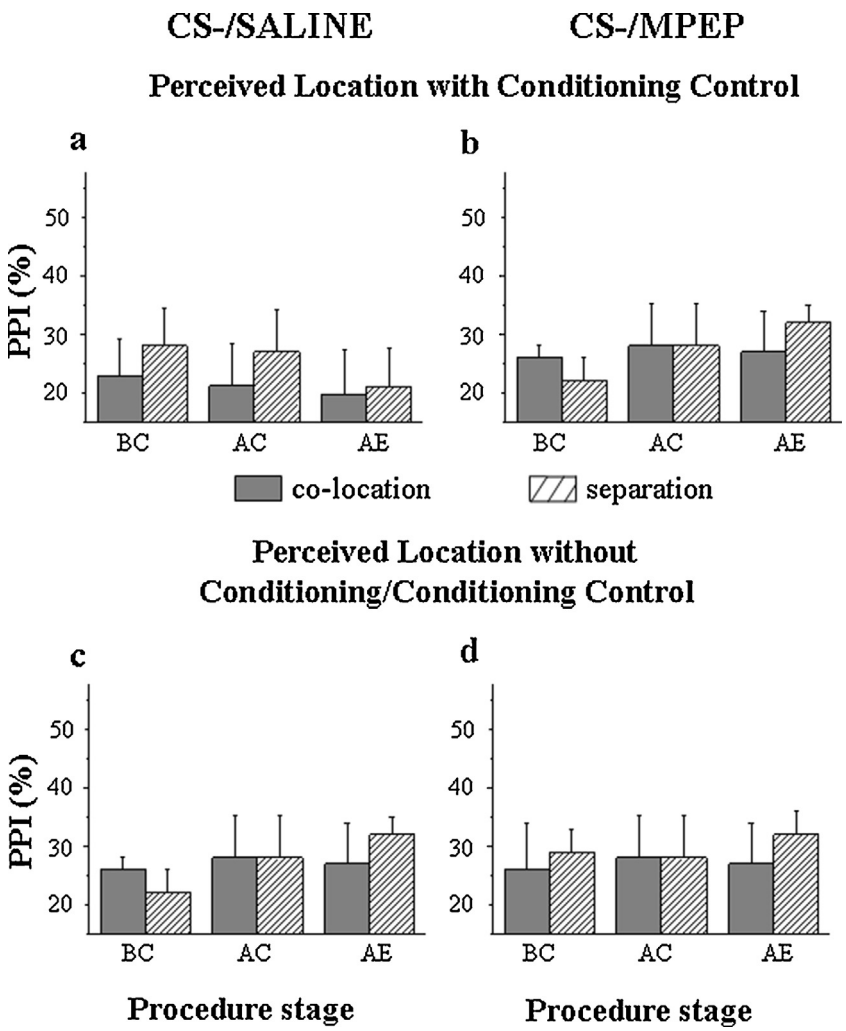

Fig. 4. Amplitudes of PPI in the two CS− groups at different procedure stages. See Fig. 3 for legend explanations.

was significant ($F_{(2,22)} = 6.01$, $p < 0.01$), but neither the main effect of separation type nor the interaction was significant (both $p > 0.05$).

3.2.2. The CS+/MPEP group

For the CS+/MPEP group (Fig. 3 right panels), a 2 (perceived prepulse location) by 3 (procedure stage) by 2 (separation type) within-subject repeated-measures ANOVA showed that all the main effects were significant (all $F > 5.33$, $p < 0.05$), the three-way interaction was significant ($F_{(2,22)} = 3.48$, $p < 0.05$), and the interaction between procedure stage and separation type was significant ($F_{(2,22)} = 6.07$, $p < 0.01$).

A further separate 3 (procedure stage) by 2 (separation type) within-subject repeated-measures ANOVAs showed that when the prepulse was perceived as coming from the location with conditioning (Fig. 3b), all the interaction and the main effects were significant (for all $F > 9.1$, $p < 0.05$). Further post hoc tests showed that (1) PPI at procedure stage BC was not affected by separation type ($p > 0.05$); (2) PPI was markedly enhanced by the conditioning manipulation (at procedure stage AC, $p < 0.05$) and the effect of separation type was significant after conditioning ($t_{(11)} = 5.07$, $p < 0.05$); (3) at procedure stage AE (with MPEP being injected 30 min before the extinction-learning manipulation), the PPI level was still significantly larger than that at procedure stage BC ($p < 0.05$) but was not significantly different from that at procedure stage AC ($p > 0.05$). Also, the effect of separation types remained significant ($t_{(11)} = 3.43$, $p < 0.05$). Thus, the extinction learning effect was abolished by MPEP injection.

When the prepulse was perceived as coming from the perceived location without conditioning (Fig. 3d), only the main effect of procedure stage was significant ($F_{(2,22)} = 11.76$, $p < 0.05$).

3.3. Modulation of PPI in the conditioning-control groups

Fig. 4 shows the PPI levels in rats with conditioning-control manipulation (CS− was unpaired with the footshock), receiving either saline (left panels) or MPEP (right panels) injection. PPI was affected by neither the conditioning-control manipulation nor the injection of saline or MPEP. For the saline group (Fig. 4a and c) and the MPEP group (Fig. 4b and d), separate 2 (prepulse location) by 3 (procedure stage) by 2 (separation type) within-subject ANOVAs showed that the main effect of prepulse location, the main effect of procedure stage, and the main effect of separation type were not significant (all $F < 2.8$; $p > 0.05$). Also, all the interactions were not significant (all $F < 3$; $p > 0.05$).

4. Discussion

4.1. Startle enhancement

Consistent with previous studies [13,16,17], this study confirms that following either the conditioning manipulation or the conditioning-control manipulation (procedure stage AC), the startle amplitude to the startling stimulus alone became enhanced

significantly, without showing manipulation type specificity. The enhanced startle response may be associated with sustained fear and/or anxiety after electrical footshock [13]. In this study, the fear extinction manipulation reduced the startle responses to the baseline level, and the extinction effect is mGluR5 dependent, supporting the view that the startle reflex is sensitive to many factors, including stress and fear [31].

4.2. Fear conditioning-induced PPI enhancement exhibits no location specificity

The PPI level is determined by the salience and processing depth of the prepulse signal [32–35]. Fear conditioning of a prepulse in rats produces the ecological significance of the prepulse, improves the salience and processing depth of the prepulse, and facilitates rats' attention to the prepulse, thereby enhancing PPI [18–20]. Importantly, the fear conditioning-induced PPI enhancement is prepulse-feature specific [13,17].

In this study, the prepulse was presented by each of the two spatially separated loudspeakers. Due to the precedence effect, the image of the prepulse was perceived as coming from one of the loudspeakers. During the fear-conditioning manipulation, the conditioned stimulus (CS+) was the prepulse delivered by the two separated loudspeakers only with one loudspeaker leading: the prepulse that was perceived as coming only from a particular location (either the left or right loudspeaker) was fear conditioned. After the unilateral conditioning, although PPI was remarkably enhanced, consistent with previous reports that fear conditioning of the prepulse enhances PPI [13,17–20], this conditioning-induced PPI enhancement (relative to the PPI level before conditioning) was not perceived-location specific: PPI was enhanced regardless of whether the conditioned prepulse was perceived at the location being conditioned or the location without conditioning. Since the fear conditioning-induced PPI enhancement is based on the function of the amygdala [17], the absence of the spatial location specificity suggests that the function of the amygdala is not sufficient to mediate the spatial specificity.

4.3. Perceived separation-induced PPI enhancement exhibits the location specificity

Previous studies have also shown that when a noise masker is present, the precedence-effect-based perceived spatial separation between the conditioned prepulse (but not the conditioning-control prepulse) and the noise masker causes an additional enhancement of PPI [13,16,17] and exhibits a marked prepulse-feature specificity [13,17]. In these previous studies, however, during the fear-conditioning manipulation, the CS+ was the prepulse stimulus delivered by each of the two spatially separated loudspeakers with balanced left/right leading, making it impossible to induce any perceived-location specificity for either conditioning-induced or perceived separation-induced PPI enhancement.

In this study, after the prepulse image perceived at a particular location was fear conditioned, perceptually spatial separation between the conditioned prepulse and the masker further enhanced PPI (relative to the PPI level when the prepulse and masker were perceived as co-located) only when the prepulse was perceived as coming from the location with conditioning (but not the location without conditioning), showing that the perceptual separation-induced PPI enhancement exhibits a marked location specificity. Thus, in addition to the stimulus-feature specificity [13,17], this study for the first time provides evidence confirming that a perceptual location specificity can be induced in top-down enhancement of PPI. Since the posterior parietal cortex (PPC) plays a role in mediating the perceptual separation-induced PPI enhancement [17], future studies should examine whether this spatial specificity is based on the function of PPC.

Based on this and previous studies [13,17], we propose that fear conditioning of a prepulse perceived at a particular location can simultaneously induce two types of conditionings, which are associated with different PPI enhancements: (1) The conditioning of (non-spatial) features of a prepulse, which is associated with the conditioning-induced PPI enhancement without the perceived location specificity, and (2) conditioning of a particular perceived prepulse location, which is associated with the perceptual separation-induced PPI enhancement with the perceived location specificity. In other words, in addition to a non-spatial feature selecting effect, conditioning of the perceived prepulse location induces a spatially gating effect, leading to that interactions of "what" and "where" processes occur in top-down modulation of PPI.

4.4. Vulnerability to extinction learning

The results of this study showed that both the startle enhancement and the PPI enhancements (including conditioning-induced and perceptual-separation-induced PPI enhancements) were completely eliminated by the extinction-learning manipulation, and all the extinction-learning effects were abolished by systemic injection of the selective antagonist of mGluR5, MPEP. Thus, the extinction learning effect is mGluR5 dependent and exhibits no specificities in manipulation, stimulus feature, or perceived location.

It is also of interest to know whether the extinction of various PPI enhancements and the extinction of startle enhancement share the same neural mechanisms, such as the inclusion of the auditory cortex, amygdala, and prefrontal cortex [36–38], and whether the extinction of the PPI enhancements in rats is also useful for studying posttraumatic stress disorder (PTSD) [39].

4.5. Stimulus and location specificities in top-down modulation of PPI and new animal models of schizophrenia

Spatial attention to potentially threatening stimuli is crucial for survival [40,41]. In humans, the precedence-effect-induced perceived spatial separation between target speech and masker facilitates selective spatial attention to the target signal stream and improves recognition of the target speech [24,42,43]. In rats, the perceived spatial separation also facilitates both spatial attention to the sound signal and ignorance of the masker after the sound signal becomes fear conditioned and ecologically salient [13,16,17]. Thus, the precedence-effect-induced facilitation of spatial attention reflects a spatial gating function of the central system in both humans and rats.

Impaired PPI in people with schizophrenia is more correlated with the severity of this disorder when the prepulse is attended, but not when ignored [10,15,44,45]. Also, speech recognition in people with schizophrenia is more vulnerable to masking than their healthy controls [46,47] and dysfunctions of spatial selective attention have also been reported in people of schizophrenia [26–28]. In rats, isolation rearing has been used for establishing neurodevelopmental models for studying schizophrenia [48–50], and both conditioning-induced PPI enhancement [13,16,19,20] and perceived separation-induced PPI enhancement [13,16] are more vulnerable to early social isolation than baseline PPI. Thus, the perceptual separation-induced enhancement of PPI with the perceived location specificity in rats will be useful for upgrading animal models with the high-order integration of feature-signal and space-signal processing for studying schizophrenia.

5. Summary

This study for the first time shows that the attentional modulation of PPI exhibits not only the prepulse-feature specificity but

also the prepulse-location specificity. After fear conditioning (but not unpaired conditioning control) is induced at a particular spatial location, although the conditioned prepulse draws more attention and enhances PPI, the enhanced PPI does not exhibit a perceived location specificity.

When a noise masker is present, precedence-effect-induced perceived spatial separation between the conditioned prepulse and the noise masker further facilitates spatial attention to the prepulse and enhances PPI only when the prepulse is perceived as coming from the conditioned location. Also, both conditioning-induced and perceptual separation-induced PPI enhancements are eliminated by extinction learning.

Thus, the spatially and nonspatially organized top-down modulation of PPI refine the complexity of PPI functions and make the gating process more flexible to the environment. It is of interest and importance in the future to investigate the application of this paradigm of attentional modulation of PPI in studies of mental disorders, such as schizophrenia.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (30950030), the 2012 Key Discipline Construction of Ethnic Medicine, the Chinese State Scholarship Fund, and "985" grants from Peking University for Physiological Psychology. The authors thank Yi Du and Chao Wang for technical supervision.

References

[1] Yeomans JS, Li L, Scott BW, Frankland PW. Tactile, acoustic and vestibular systems sum to elicit the startle reflex. Neurosci Biobehav Rev 2002;26:1–11.
[2] Hoffman HS, Ison JR. Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. Psychol Rev 1980;87:175–89.
[3] Foss JA, Ison JR, Torre JP, Wansack S. The acoustic startle response and disruption of aiming: II. Modulation by forewarning and preliminary stimuli. Hum Factors 1989;31:319–33.
[4] Hoffman HS, Searle JL. Acoustic variables in the modification of startle reaction in the rat. J Comp Physiol Psychol 1965;60:53.
[5] Graham F. The more or less startling effects of weak prestimulation. Psychophysiology 1975;12:238–48.
[6] Davis M, Gendelman PM. Plasticity of the acoustic startle response in the acutely decerebrate rat. J Comp Physiol Psychol 1977;91:549.
[7] Li L, Frost BJ. Azimuthal sensitivity of rat pinna reflex: EMG recordings from cervicoauricular muscles. Hear Res 1996;100:192–200.
[8] Fendt M, Li L, Yeomans JS. Brain stem circuits mediating prepulse inhibition of the startle reflex. Psychopharmacology (Berl) 2001;156:216–24.
[9] Schell AM, Dawson ME, Hazlett EA, Filion DL. Attentional modulation of startle in psychosis-prone college students. Psychophysiology 1995;32:266–73.
[10] Hazlett EA, Romero MJ, Haznedar MM, New AS, Goldstein KE, Newmark RE, et al. Deficient attentional modulation of startle eyeblink is associated with symptom severity in the schizophrenia spectrum. Schizophr Res 2007;93:288–95.
[11] Hazlett EA, Levine J, Buchsbaum MS, Silverman JM, New A, Sevin EM, et al. Deficient attentional modulation of the startle response in patients with schizotypal personality disorder. Am J Psychiatry 2003;160:1621–6.
[12] Li L, Du Y, Li N, Wu X, Wu Y. Top-down modulation of prepulse inhibition of the startle reflex in humans and rats. Neurosci Biobehav Rev 2009;33:1157–67.
[13] Du Y, Wu X, Li L. Emotional learning enhances stimulus-specific top-down modulation of sensorimotor gating in socially reared rats but not isolation-reared rats. Behav Brain Res 2010;206:192–201.
[14] Dawson ME, Hazlett EA, Filion DL, Nuechterlein KH, Schell AM. Attention and schizophrenia: impaired modulation of the startle reflex. J Abnorm Psychol 1993;102:633–41.
[15] Dawson ME, Schell AM, Hazlett EA, Nuechterlein KH, Filion DL. On the clinical and cognitive meaning of impaired sensorimotor gating in schizophrenia. Psychiatry Res 2000;96:187–97.
[16] Du Y, Li JY, Wu XH, Li L. Precedence-effect-induced enhancement of prepulse inhibition in socially reared but not isolation-reared rats. Cogn Affect Behav Neurosci 2009;9:44–58.
[17] Du Y, Wu X, Li L. Differentially organized top-down modulation of prepulse inhibition of startle. J Neurosci 2011;31:13644–53.
[18] Huang J, Yang Z, Ping J, Liu X, Wu X, Li L. 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 2007;223:1–10.
[19] Li NX, Ping J, Wu R, Wang C, Wu X, Li L. Auditory fear conditioning modulates prepulse inhibition in socially reared rats and isolation-reared rats. Behav Neurosci 2008;122:107–18.
[20] Zou D, Huang J, Wu X, Li L. Metabotropic glutamate subtype 5 receptors modulate fear-conditioning induced enhancement of prepulse inhibition in rats. Neuropharmacology 2007;52:476–86.
[21] Li L, Qi JG, He Y, Alain C, Schneider BA. Attribute capture in the precedence effect for long-duration noise sounds. Hear Res 2005;202:235–47.
[22] Litovsky RY, Colburn HS, Yost WA, Guzman SJ. The precedence effect. J Acoust Soc Am 1999;106:1633–54.
[23] Wallach H, Newman EB, Rosenzweig MR. The precedence effect in sound localization. J Acoust Soc Am 1949;62:315–36.
[24] Li H, Kong L, Wu X, Li L. Primitive auditory memory is correlated with spatial unmasking that is based on direct-reflection integration. PLoS ONE 2013;8:e63106.
[25] Du Y, Wang Q, Zhang Y, Wu X, Li L. Perceived target–masker separation unmasks responses of lateral amygdala to the emotionally conditioned target sounds in awake rats. Neuroscience 2012;225:249–57.
[26] Dalmaso M, Galfano G, Tarqui L, Forti B, Castelli L. Is social attention impaired in schizophrenia? Gaze, but not pointing gestures, is associated with spatial attention deficits. Neuropsychology 2013;27:608.
[27] Park S, Holzman PS. Schizophrenics show spatial working memory deficits. Arch Gen Psychiatry 1992;49:975.
[28] Park S, Püschel J, Sauter BH, Rentsch M, Hell D. Spatial selective attention and inhibition in schizophrenia patients during acute psychosis and at 4-month follow-up. Biol Psychiatry 2002;51:498–506.
[29] Maren S. Seeking a spotless mind: extinction, deconsolidation, and erasure of fear memory. Neuron 2011;70:830–45.
[30] Xu J, Zhu Y, Contractor A, Heinemann SF. mGluR5 has a critical role in inhibitory learning. J Neurosci 2009;29:3676–84.
[31] Vrana SR, Calhoun PS, McClernon FJ, Dennis MF, Lee ST, Beckham JC. Effects of smoking on the acoustic startle response and prepulse inhibition in smokers with and without posttraumatic stress disorder. Psychopharmacology (Berl) 2013;230:477–85.
[32] Carlson S, Willott JF. The behavioral salience of tones as indicated by prepulse inhibition of the startle response: relationship to hearing loss and central neural plasticity in C57BL/6J mice. Hear Res 1996;99:168–75.
[33] Franklin JC, Moretti NA, Blumenthal TD. Impact of stimulus signal-to-noise ratio on prepulse inhibition of acoustic startle. Psychophysiology 2007;44:339–42.
[34] Ison JR, Bowen GP, Pak J, Gutierrez E. Changes in the strength of prepulse inhibition with variation in the startle baseline associated with individual differences and with old age in rats and mice. Psychobiology 1997;25:266–74.
[35] Röskam S, Koch M. Enhanced prepulse inhibition of startle using salient prepulses in rats. Int J Psychophysiol 2006;60:10–4.
[36] Falls W, Miserendino M, Davis M. Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. J Neurosci 1992;12:854–63.
[37] Milad MR, Quirk GJ. Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 2002;420:70–4.
[38] Quirk GJ, Armony JL, LeDoux JE. Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. Neuron 1997;19:613–24.
[39] Adamec R. Transmitter systems involved in neural plasticity undelying increased anxiety and defense—implications for understanding anxiety following traumatic stress. Neurosci Biobehav Rev 1997;21:755–65.
[40] Bertels J, Kolinsky R, Coucke D, Morais J. When a bang makes you run away: spatial avoidance of threatening environmental sounds. Neurosci Lett 2013;535:78–83.
[41] Fritz JB, Elhilali M, David SV, Shamma SA. Auditory attention—focusing the searchlight on sound. Curr Opin Neurobiol 2007;17:437–55.
[42] Freyman RL, Clifton RK, Litovsky RY. Dynamic processes in the precedence effect. J Acoust Soc Am 1991;90:874.
[43] Li L, Daneman M, Qi JG, Schneider BA. Does the information content of an irrelevant source differentially affect speech recognition in younger and older adults. J Exp Psychol Hum Percept Perform 2004;30:1077–91.
[44] Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl) 2001;156:234–58.
[45] Swerdlow NR, Weber M, Qu Y, Light GA, Braff DL. Realistic expectations of prepulse inhibition in translational models for schizophrenia research. Psychopharmacology (Berl) 2008;199:331–88.
[46] Wu C, Cao SY, Zhou FC, Wang CY, Wu XH, Li L. Masking of speech in people with first-episode schizophrenia and people with chronic schizophrenia. Schizophr Res 2012;134:33–41.
[47] Wu C, Li HH, Tian Q, Wu XH, Wang CY, Li L. Disappearance of the unmasking effect of temporally pre-presented lipreading cues on speech recognition in people with chronic schizophrenia. Schizophr Res 2013;150:594–5.
[48] Weiss IC, Domeney AM, Heidbreder CA, Moreau J-L, Feldon J. Early social isolation, but not maternal separation, affects behavioral sensitization to amphetamine in male and female adult rats. Pharmacol Biochem Behav 2001;70:397–409.
[49] Weiss IC, Domeney AM, Moreau J-L, Russig H, Feldon J. Dissociation between the effects of pre-weaning and/or post-weaning social isolation on prepulse inhibition and latent inhibition in adult Sprague-Dawley rats. Behav Brain Res 2001;121:207–18.
[50] Weiss IC, Iorio LD, Feldon J, Domeney AM. Strain differences in the isolation-induced effects on prepulse inhibition of the acoustic startle response and on locomotor activity. Behav Neurosci 2000;114:364–73.