



Research report

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



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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.

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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.

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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.

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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 $24 \pm 2^\circ\text{C}$ 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

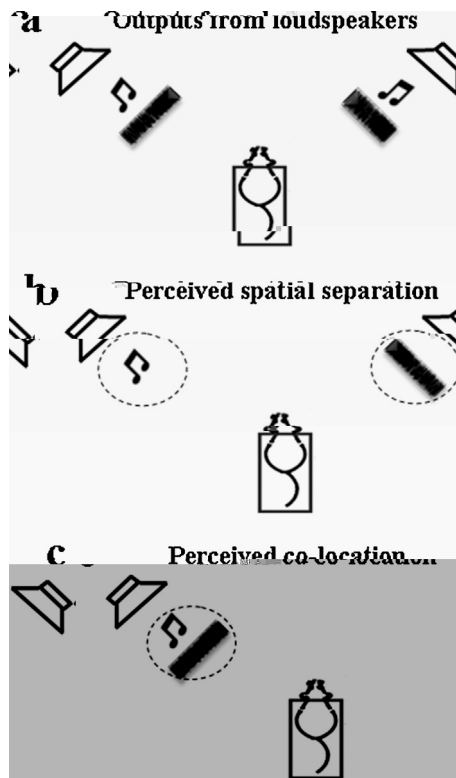


Fig. 1. Diagrams showing the physical (Panel a) and perceived (Panels b and c) spatial relationship between the prepulse (the music note) and the noise masker (noise waveform). (Panel a) Both the prepulse and the noise masker were delivered by each of the two horizontally separated loudspeakers. (Panel 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

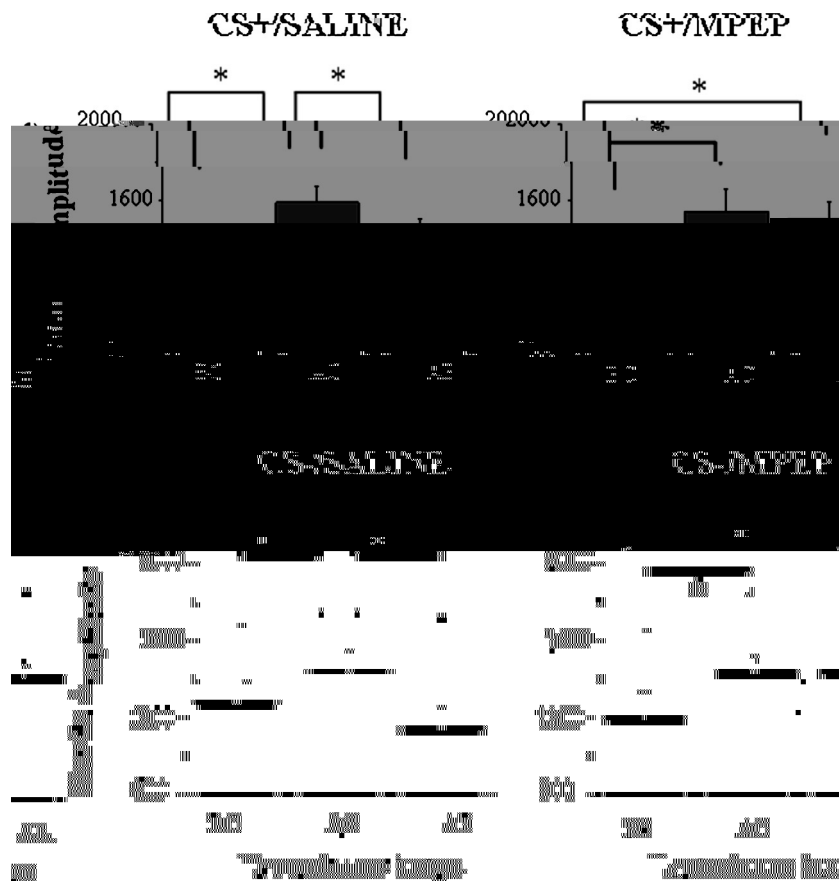


Fig. 2. Amplitudes of startling responses to the startling stimulus alone in each of the four rat groups. CS+, the prepulse that was fear conditioned; CS-, the prepulse that was control-conditioned; SALINE, saline injection; MPEP, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) injection; BC, before conditioning;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

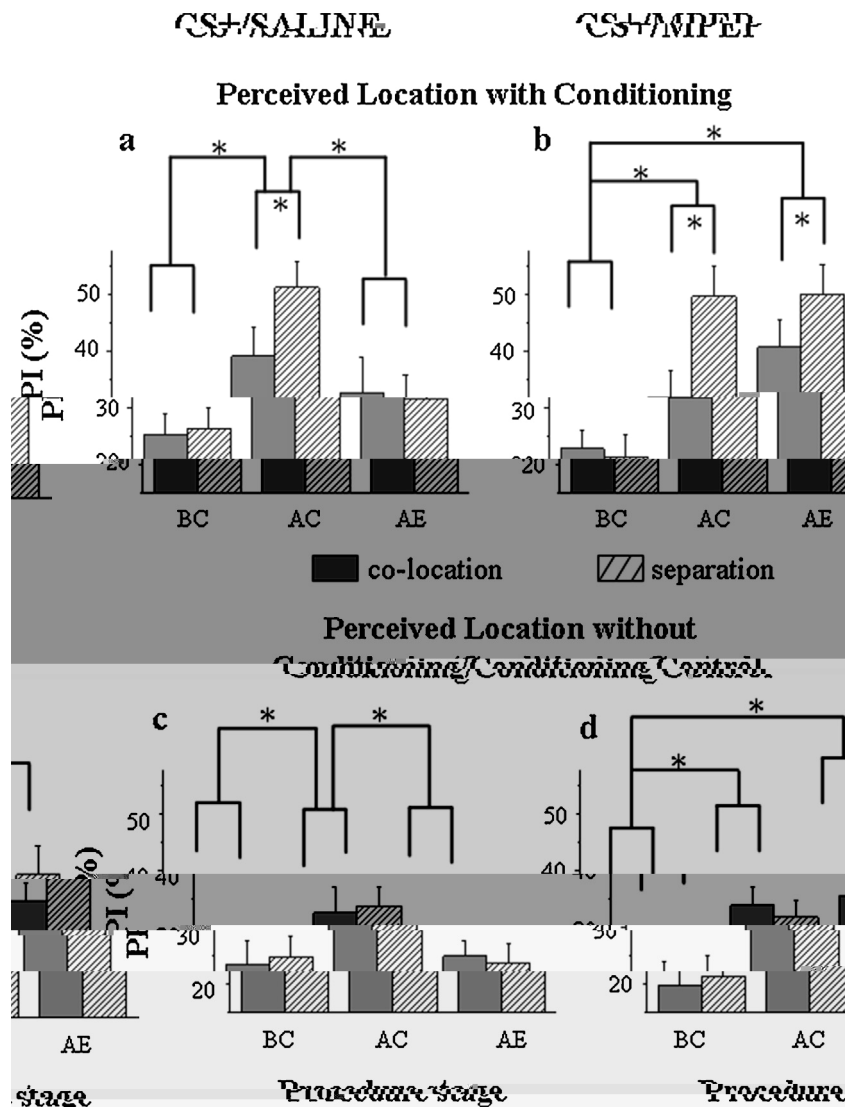


Fig. 3. Amplitudes of PPI in the two CS+ groups at different procedure stages. The top two panels present the PPI levels when the prepulse was perceived at the location with conditioning, and bottom two panels present the PPI levels when the prepulse was perceived from the perceived location without 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

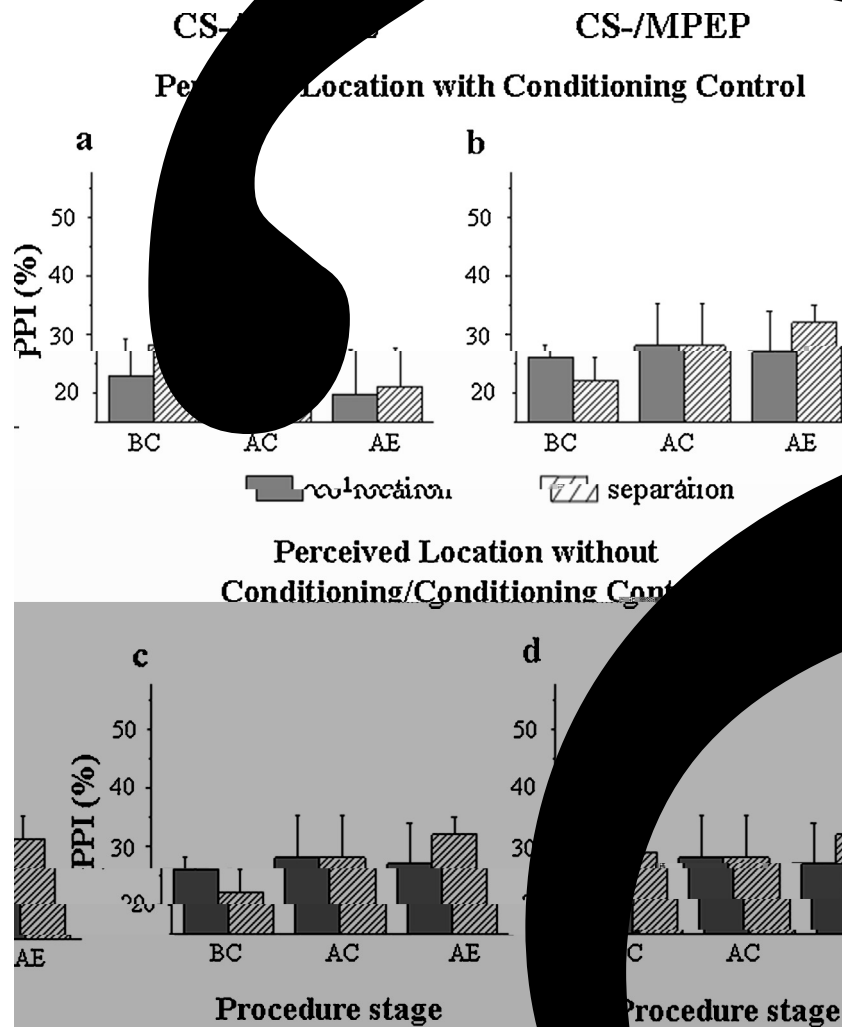


Fig. 4. Amplitudes of PPI in the two CS- groups at different procedure stages. See Fig. 3 for legend explanation.

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 location without conditioning (Fig. 3c), 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.

3.3. Modulation of PPI by 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

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 (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 perceptual location specificity.

When a noise masker is present, precedence-effect-induced perceived spatial separation between the conditioned prepulse and the noise masker further facilitates