Metabotropic glutamate subtype 5 receptors modulate fear-conditioning induced enhancement of prepulse inhibition in rats

Dan Zou [a], Juan Huang [a], Xihong Wu [a], Liang Li [a,b,*]

[a] *Department of Psychology, National Key Laboratory on Machine Perception, Speech and Hearing Research Center, Peking University, Beijing 100871, China*
[b] *Department of Psychology, Center for Research on Biological Communication Systems, University of Toronto at Mississauga, Mississauga, Ont. L5L 1C6, Canada*

Abstract

Non-startling acoustic events presented shortly before an intense startling sound can inhibit the acoustic startle reflex. This phenomenon is called prepulse inhibition (PPI), and is widely used as a model of sensorimotor gating. The present study investigated whether PPI can be modulated by fear conditioning, whose acquisition can be blocked by the specific antagonist of metabotropic glutamate receptors subtype 5 (mGluR5), 2-methyl-6-(phenylethynyl)-pyridine (MPEP). The results show that a gap embedded in otherwise continuous noise sounds, which were delivered by two spatially separated loudspeakers, could inhibit the startle reflex induced by an intense sound that was presented 50 ms after the gap. The inhibitory effect depended on the duration of the gap, and was enhanced by fear conditioning that was introduced by temporally pairing the gap with footshock. Intraperitoneal injection of MPEP (0.5 or 5 mg/kg) 30 min before fear conditioning blocked the enhancing effect of fear conditioning on PPI, but did not affect either the baseline startle magnitude or PPI if no fear conditioning was introduced. These results indicate that PPI is enhanced when the prepulse signifies an aversive event after fear conditioning. Also, mGlu5Rs play a role in preserving the fear-conditioning-induced enhancement of PPI.
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1. Introduction

The neural substrate of suppressing irrelevant sensory information to ensure useful sensory information processing is called sensory gating. Impaired sensory gating in schizophrenic patients has been assumed to cause thought disorder and emotion abnormality (for reviews see Braff et al., 2001; Geyer et al., 2001; Swerdlow et al., 2001; van den Buuse et al., 2005; Weiss and Feldon, 2001). The startle reflex is the strongest whole-body reflective response (Landis and Hunt, 1939). It can be elicited by intense sensory stimuli with several important features, such as short latency, potent summation, and wide dynamic range (Li and Frost, 1996; Li and Yeomans, 1999; Li et al., 2001). The neural circuit mediating startle is short, and the key structure is the caudal pontine reticular nucleus, in which the giant neurons receive axonal projections from the cochlear nucleus, trigeminal nucleus and vestibular nucleus, and send projections to motor areas of cranial nerve nuclei (e.g. motor neurons in facial nerve nucleus) and the spinal cord (for reviews see Koch and Schnitzler, 1997; Yeomans et al., 2002). The startle reflex is the fast response to threatening stimuli and important for adaptation to the environment, but also has a disruptive effect on cognitive/behavioral performances. For example, the acoustic startle reflex can disrupt perception/motor tasks in humans (Foss et al., 1989) and learned lever-pressing behaviors in rats (Hoffman and Overman, 1971). However, the

central nervous system also has neural circuits of inhibiting startle to reduce the disruptive influence to cognition and behavior. Prepulse inhibition (PPI) of the startle reflex is the normal reduction of the startle reflex to an intense startling stimulus when this startling stimulus is shortly preceded by a weaker sensory stimulus (prepulse), and widely recognized as a cross-species model of sensorimotor gating (Braff and Geyer, 1990; Graham, 1975; Hoffman and Ison, 1980; Ison and Hoffman, 1983; Li and Yue, 2002).

Graham proposed a ''protection-of-processing'' theory for justifying PPI (Graham, 1975): a weak prepulse stimulus followed by an intense stimulus can trigger not only the information processing for the prepulse signal but also a gating mechanism that dampens the information of the intense disruptive stimulus, therefore protects the early process of the prepulse stimulus. This proposal has been supported by several lines of research using human subjects. First, Foss et al. (1989) found that presentation of a weak acoustic stimulus 100 ms prior to a startle-eliciting stimulus significantly reduces startle-produced errors in an aiming task. In addition, the accuracy of discriminating the prepulse stimulus is highly correlated to the degree of suppression of the startle reflex (Filion and Ciranni, 1994; Mussat-Whitlow and Blumenthal, 1997; Norris and Blumenthal, 1995, 1996; Perlstein et al., 1989, 1993). Finally, the startling sound is perceived as less intense when it is preceded by a prepulse sound (Blumenthal et al., 1996; Perlstein et al., 1993). Thus, PPI of the startle reflex reflects activation of a protective mechanism in the central nervous system.

PPI can be modulated in animals by manipulations of neural activity of various forebrain structures, including the amygdala (Bouwmeester et al., 2002; Daenen et al., 2003; Decker et al., 1995; Fendt et al., 2000; Stevenson and Gratton, 2004; Wan and Swerdlow, 1997; for reviews see Li and Shao, 2003; Swerdlow et al., 2001). For example, either large lesions of the amygdala or focal lesions of the basolateral amygdala significantly reduce PPI (Decker et al., 1995; Wan and Swerdlow, 1997). It has been well known that the lateral nucleus of the amygdala (LA) mediates auditory fear conditioning (AFC) (Hitchcock and Davis, 1986; Romanski and LeDoux, 1992; Maren, 1996; Fendt, 2001; Goosens and Maren, 2001; Tazumi and Okaichi, 2002). Auditory inputs to LA originate mainly from the medial geniculate nucleus (MGN) and auditory association cortex (AAC) (LeDoux et al., 1990; Turner and Herkenham, 1991; Romanski and LeDoux, 1993; Mascagni et al., 1993; Doron and LeDoux, 1999; Woodson et al., 2000). Interestingly, the amygdala also plays a role in developing neuronal plasticity in the MGN during AFC (Maren et al., 2001; Poremba and Gabriel, 2001). The MGN has been suggested to be an auditory structure that modulates PPI (Zhang et al., 1999). It would be intriguing and important to know whether AFC can have certain influence to PPI. When a prepulse stimulus becomes a signal informing aversive events following AFC, does it grow to be more potent in inhibiting the startle reflex? However, to our knowledge, this issue has not been investigated before.

Metabotropic glutamate receptors (mGluRs) are coupled to various second messenger cascades, and involved in synaptic plasticity associated with learning and memory (for reviews see Riedel, 1996; Simonyi et al., 2005). The group I metabotropic glutamate receptors subtype 5 (mGluR5) are critical for formation of AFC (Schulz et al., 2001; Fendt and Schmid, 2002; Lee et al., 2002; Rodrigues et al., 2002). Some studies have confirmed that systemic administration of 2-methyl-6-(phenylethynyl)-pyridine (MPEP), the non-competitive, selective, and systemically active antagonist of mGluR5 (Gasparini et al., 1999), does not effect either PPI or the acoustic startle reflex (Henry et al., 2002; Kinney et al., 2003; Schulz et al., 2001; Spooren et al., 2000). For example, Schulz et al. (2001) have reported that oral administration of MPEP did not affect PPI and the magnitude of the acoustic startle reflex at the dosage of 3.0 mg/kg, short-term habituation of startle at the dosage of 0.3, 3.0, or 30.0 mg/kg, and sensitization of startle by footshock at the dosage of 3.0 mg/kg. Therefore, MPEP would be an ideal pharmacological agent used for studying the modification of PPI by AFC.

This study was to investigate whether PPI, the model of sensorimotor gating, can be modified by AFC, which is induced by explicitly pairing the prepulse stimulus with footshock. In addition, this study was also to investigate whether MPEP affects the effect of AFC on PPI. The prepulse stimulus used in the present study was a gap (a transient drop in sound level) embedded in otherwise continuous background noise sounds (Leitner and Girten, 1997; Barsz et al., 1998, 2002; Ison et al., 1998, 2002; Ison and Bowen, 2000).

2. Methods

2.1. Animals

Fifty-three young adult male albino Sprague—Dawley rats (weighted between 160—180 g), provided by Beijing Vital River Experimental Animals Technology Ltd., were used in this study. They were housed individually in plastic cages and placed on a 12 h light/dark cycle, with food and water freely available. These male rats used for this study had become adults before they were purchased. They did not experience social isolation during their early ages. They were allowed at least 1 week to adapt to the experimental environment before testing. During testing, they were randomly divided into the following 6 groups: (1) AFC only (10 rats); (2) AFC control (9 rats); (3) AFC/saline injection (11 rats); (4) AFC/0.5 mg/kg MPEP injection (8 rats); (5) AFC/5 mg/kg MPEP injection (8 rats); and (6) 5 mg/kg MPEP injection only (7 rats).

All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experiments were carried out in according with the guidelines of the Canadian Council of Animal Care.

2.2. Apparatus

The rat's whole-body startle reflex, which was induced by an intense 10-ms broadband noise burst (100 dB SPL) that was delivered by a loudspeaker 30 cm 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. The scale had a platform, on which a specially designed small metal-mesh cage for restraining the rat was placed. There were three different cage sizes for tested rats with different body weights. The internal dimensions of the three types of cages were: (1) large cage: length = 151 mm, width = 58 mm, and height = 51 mm; (2) medium cage: length = 139 mm, width = 52 mm, and height = 44 mm; and (3) small cage: length = 131 mm, width = 48 mm, and height = 40 mm. The

platform had a flexible piezoelectric film material laminated to the bottom, which generated voltages proportional to the magnitude of the rat's acoustic startle reflex. This voltage was amplified and passed through an analog/digital-digital/analog converter. A Pentium IV microcomputer was used to run the experimental programs, which were custom-developed by the National Key Laboratory on Machine Perception, Peking University. Startle-induced electrical voltages were sampled at a frequency of 16 kHz for 500 ms, beginning with the onset of the startling stimulus. Peak values during this interval were digitized and measured.

Two additional high-frequency loudspeakers, which were placed on the azimuthal plane in the frontal field with a 100° separation angle, was 52 cm away from the rat's head position (Fig. 1). These two loudspeakers delivered continuous and independent broadband noise sounds (55 dB SPL) as background stimuli. They were also used to deliver gaps as prepulse stimuli. Sound levels were calibrated using a B&K sound level meter (Type 2230) whose microphone was placed at the central location of the rat's head when the rat was absent, using a "Fast"/"Peak" meter response.

During AFC, an electrical current stimulator (Grass Model S88K) was used to produce electric shock stimuli through two small pieces of platinum slices fixed to the back of one of the rat's hindpaws. Timing of sound stimuli and that of footshock were also controlled by the computer.

2.3. Adaptation

The rat was placed into the cage with its head extending out of the cage. The restrained rat was exposed to acoustic stimuli used for PPI testing (see below) 20 min per day for 3 successive days. The purpose of this pre-testing procedure was to allow the rat to become adapted to the experimental environments.

2.4. Baseline prepulse inhibition

PPI baseline of animals was measured on the fourth day of testing. Before testing, the rat was placed into the cage for 5 min with the startling stimulus being presented repeatedly without gap presentation. During testing, a gap was presented from each of the loudspeakers without inter-loudspeaker delay. Fifty ms after the end of the gap, the intense startling noise burst was presented by the top loudspeaker. About 30 s after the end of the gap, a new trial began. The inter-trial interval varied between 25 s and 35 s with the mean of 30 s in a random fashion. There were 7 different gap sizes (0, 5, 10, 20, 40, 80, and 160 ms), and each was presented 10 times in a testing session. The order of presenting the gaps of different sizes was in a random fashion.

Twenty-four hrs after conditioning and/or injection (see below), post-treatment PPI was measured using the same procedures.
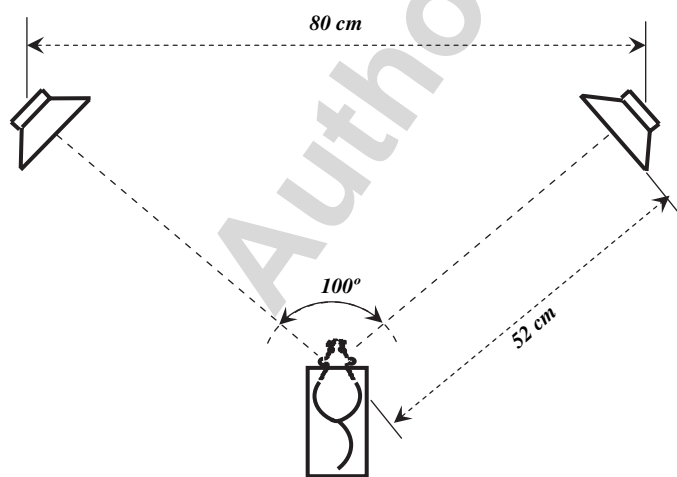

Fig. 1. Diagram showing the spatial arrangement of the two horizontal loudspeakers, which delivered the prepulse gap, and the rat's body position.

2.5. Fear conditioning

On the fifth day, all the rat groups, except the group with 5 mg/kg MPEP injection only, went through the fear conditioning procedures. During AFC, the acoustic conditioned stimulus (CS) was a 100-ms gap delivered by each of the horizontal loudspeakers. Based on the study by Sikes and Vogt (1992) and that by Villanueva et al. (1989), the electrical unconditioned stimulus (US) used in the present study was 6-mA rectangular-pulse (pulse duration = 3 ms) footshock provided by a Grass S-88 stimulator (Grass, Quincy, MA, USA) via a constant-current, photoelectric stimulus-isolation units (model PSIU6). For the following 4 rat groups, 20 precisely combined pairs of CS and US (footshock started 3 ms before the gap ending, and co-terminated with the gap) were presented with the repetition rate around 30 s: (1) AFC; (2) AFC/saline injection; (3) AFC/0.5 mg/kg MPEP injection; and (4) AFC/5 mg/kg MPEP injection.

For the AFC control group, the pairing of CS and US was in a randomly temporal manner.

2.6. Drug injection

Also on the fifth day, MPEP ($C_{14}H_{11}N \cdot HCl$, Sigma-Aldrich Corporate, St Louis, MO, USA) solution was freshly prepared with 0.9% saline and administered intraperitoneally 30 min before conditioning in the following 3 groups: (1) AFC/saline injection (0 mg/kg MPEP); (2) AFC/0.5 mg/kg MPEP injection; and (3) AFC/5 mg/kg MPEP injection. For rats in the group of 5 mg/kg MPEP injection only, they did not receive AFC. The injection volume for each of the injected rats was 1 ml.

2.7. Post-treatment testing of prepulse inhibition

On the sixth day, PPI of startle was tested again for all the rat groups.

2.8. Statistical analysis

To make results of treatments comparable across animals, prepulse-inhibited responses for each animal were normalized relative to the individual's response to the startling sound alone, and the percent response data were used in the ANOVAs. The following equation was used to calculate the percent response:

Percent response $= 100\% \times$ (amplitude to startling sound preceded by prepulse/ amplitude to startling sound alone)

To test the effects of AFC, combined AFC and MPEP injection, or MPEP injection alone on PPI, a 7 (gap size) by 2 (before and after treatment) two-way within-group ANOVA was used for each group in each experiment.

In addition, to compare the differences between the following 3 groups, a 3 (group) by 7 (gap size) two-way mixed between-and-within-group ANOVA was used: (1) AFC/saline injection; (2) AFC/0.5 mg/kg MPEP injection; and (3) AFC/5 mg/kg MPEP injection.

The analyses were performed using SPSS 13.0 software. The null-hypothesis rejection level was set at 0.05.

3. Result

3.1. Mean prepulse inhibition across 53 rats before treatments

In each of the 53 rats used in the present study, the intense 10-ms noise burst could reliably elicit whole-body startle responses, whose latencies of primary peak components were about 15 ms after the onset of the startling noise burst (Fig. 2).

For all the 53 rats, compared to the startle responses under the zero-gap condition (gap duration = 0 ms), startle responses,
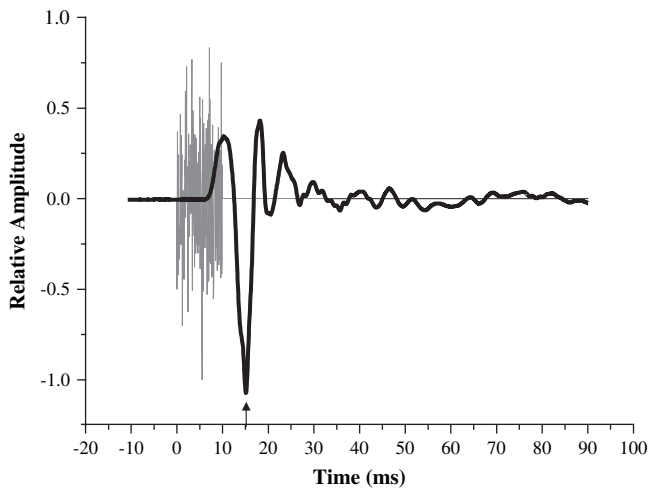
Fig. 2. A representative waveform of the startle response to the startling noise burst when the gap size was zero. The primary peak component is indicated by the arrow. The temporal position of the startling noise burst is also indicated. The onset of the startling noise burst is the time zero in the figure for determining the latency of the primary peak of the startle response.

which were measured on the fourth day, were significantly inhibited by gap presentations at each of the 6 gap-size conditions (5 ms: $F_{1,104} = 4.804$, $p < 0.05$; 10 ms: $F_{1,104} = 50.722$, $p < 0.05$; 20 ms: $F_{1,104} = 203.148$, $p < 0.05$; 40 ms: $F_{1,104} = 268.730$, $p < 0.05$; 80 ms: $F_{1,104} = 206.986$, $p < 0.05$; 160 ms: $F_{1,104} = 261.695$, $p < 0.05$). Also, the inhibitory effect increased as the gap size increased (Fig. 3).
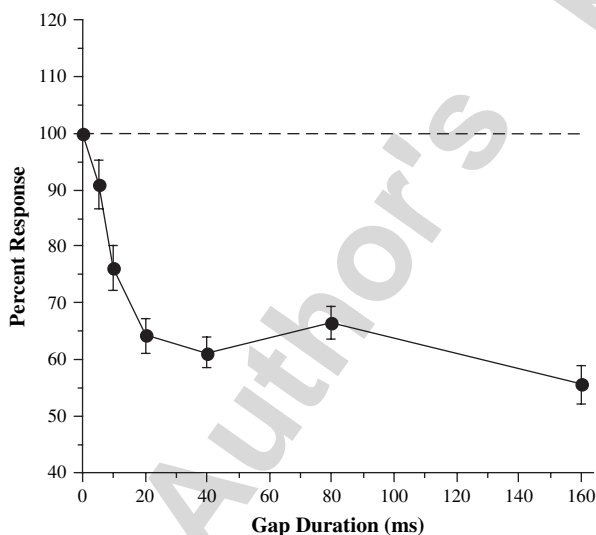

Fig. 3. The mean percent startle response across 53 rats as the function of the size of the gap that was used as the prepulse stimulus. The startle measurements for each rat were made on the fourth testing day. When the size of gap was 5, 10, 20, 40, 80, or 160 ms, the startle amplitude was significantly smaller than that when the gap size was zero (5 ms: $F_{1,104} = 4.804$, $p < 0.05$; 10 ms: $F_{1,104} = 50.722$, $p < 0.05$; 20 ms: $F_{1,104} = 203.148$, $p < 0.05$; 40 ms: $F_{1,104} = 268.730$, $p < 0.05$; 80 ms: $F_{1,104} = 206.986$, $p < 0.05$; 160 ms: $F_{1,104} = 261.695$, $p < 0.05$). The error bars indicate the standard error of the mean.

3.2. Effects of fear conditioning on prepulse inhibition

The effects of gap-footshock pairing on gap-induced PPI of the acoustic startle reflex are shown in Fig. 4. For the group of AFC (gap and footshock were precisely paired) (Fig. 4, upper panels), following precisely temporal pairing of the 100-ms gap and footshock, PPI was markedly enhanced. A 7 (gap size) by 2 (before and after pairing) two-way within-group ANOVA shows that the interaction between gap size and gap-footshock pairing was not significant ($F_{6,54} = 4.018$, $p > 0.05$), but the main effect of gap size was significant ($F_{6,54} = 13.890$, $p < 0.05$), and the main effect of gap-footshock pairing was significant ($F_{1,9} = 5.730$, $p < 0.05$). The startle responses, when the gap size was zero, were not affected by the gap-footshock pairing ($F_{1,9} = 0.001$, $p > 0.05$).

For the group of AFC control (Fig. 4, lower panels), following temporally random pairing of the 100-ms gap and footshock, PPI was not changed at most of the gap size conditions. A 7 by 2 two-way within-group ANOVA shows that the interaction between gap size and gap-footshock pairing was not significant ($F_{6,48} = 1.200$, $p > 0.05$), but the main effect of gap size was significant ($F_{6,48} = 20.100$, $p < 0.05$). However, the main effect of gap-footshock pairing was not significant ($F_{1,8} = 0.012$, $p > 0.05$). The startle responses, when the gap size was zero, were not affected by the gap-footshock pairing ($F_{1,8} = 0.020$; $p > 0.05$).

3.3. Effects of combination of fear conditioning and MPEP injection on prepulse inhibition

The effects of combination of gap-footshock pairing and MPEP injection on gap-induced PPI of the acoustic startle reflex for the following 3 groups are shown in Fig. 5: (1) AFC/ saline injection; (2) AFC/0.5 mg/kg MPEP injection; and (3) AFC/5 mg/kg MPEP injection. To analyze the effects of the experimental treatments on PPI, the following ANOVAs were used. First, the group differences before combined AFC and injection treatments were analyzed. And then, the group differences after combined AFC and injection treatments were analyzed. Finally, for each of the 3 injection groups, the within-group differences before and after the experimental treatments (Fig. 5) were analyzed.

For startle responses to the combined prepulse and startling stimuli before combined AFC and injection treatments, a 3 (group) by 7 (gap size) two-way mixed between-and-within-group ANOVA shows that the interaction between group and gap-size effects was not significant ($F_{12,40} = 1.354$, $p > 0.05$), the main effect of gap size was significant ($F_{6,19} = 29.008$, $p < 0.05$), and the main effect of group was not significant ($F_{2,24} = 1.941$, $p > 0.05$). Thus before combined AFC and injection treatments, the gap size significantly determined the PPI effect, and there were no significant differences between the 3 groups.

For startle responses to the combined prepulse and startling stimuli after combined AFC and injection treatments, a 3 (group) by 7 (gap size) two-way mixed between-and-within-group ANOVA shows that the interaction between group and
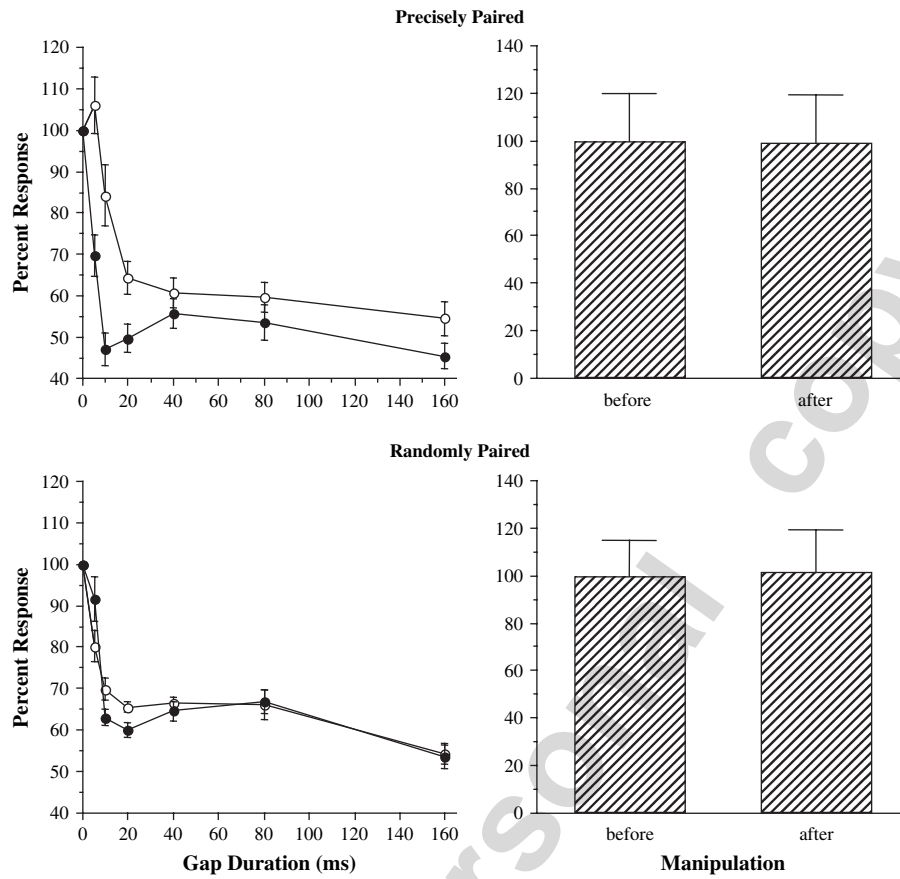
Precisely Paired

Randomly Paired

Fig. 4. Left panels: The mean startle response as a function of the duration of the prepulse gap for the group of auditory fear conditioning (AFC) only and that for the group of AFC control. Open circles, before gap-footshock pairing; filled circles, after gap-footshock pairing. For the group of AFC, both the gap-size effect and the gap-footshock-pairing effect were significant (gap size: $F_{6,54} = 13.89$; $p < 0.05$; gap-footshock pairing: $F_{1,9} = 5.73$; $p < 0.05$). For the group of AFC control, the gap-size effect was significant ($F_{6,48} = 20.10$; $p < 0.05$), but the gap-footshock-pairing effect was not significant ($F_{1,8} = 0.012$; $p > 0.05$). Right panels: The mean startle responses under zero-gap condition before and after gap-footshock pairing. For each of the two groups, the startle responses, when the gap size was zero, were not significantly affected by the gap-footshock pairing (group of AFC: $F_{1,9} = 0.001$, $p > 0.05$; group of AFC control: $F_{1,8} = 0.020$; $p > 0.05$). Error bars indicate the standard error of the mean.

gap size was not significant ($F_{12,40} = 1.284$, $p > 0.05$), the main effect of gap size was significant ($F_{6,19} = 8.386$, $p < 0.05$), and the main effect of group was also significant ($F_{2,24} = 4.438$, $p < 0.05$). Thus after combined AFC and injection treatments, the gap size still significantly determined the globe PPI effect, and there were significant differences between the 3 groups. Separate one-way ANOVAs show that there was a significant difference between the group of AFC/0.5 mg/kg MPEP injection and the group of AFC/saline injection ($F_{1,17} = 11.125$, $p < 0.05$) and between the group of AFC/5 mg/kg MPEP and the group of AFC/saline injection ($F_{1,17} = 4.754$, $p < 0.05$), but not between the group of AFC/0.5 mg/kg MPEP and the group of AFC/5 mg/kg MPEP ($F_{1,14} = 0.058$, $p > 0.05$).

We also evaluated the treatment effects using within-group ANOVAs. For the group of AFC/saline injection (0 mg/kg) (Fig. 5, top panels), following combination of AFC and saline injection, PPI was enhanced. A 7 (gap size) by 2 (before and after treatment) two-way within-group ANOVA shows that the interaction between gap size and treatment was not significant ($F_{6,60} = 3.342$, $p > 0.05$), but the main effect of gap size

was significant ($F_{6,60} = 40.91$; $p < 0.05$), and the main effect of conditioning/injection was significant ($F_{1,10} = 5.24$; $p < 0.05$). The startle responses, when the gap size was zero, were not affected by the treatment ($F_{1,10} = 3.386$, $p > 0.05$).

For the group of AFC/0.5 mg/kg MPEP injection (Fig. 5, middle panels), following combination of AFC and 0.5 mg/kg MPEP injection, PPI was reduced. A 7 by 2 two-way within-group ANOVA shows that the interaction between gap size and treatment was not significant ($F_{6,42} = 3.539$, $p > 0.05$), and the main effect of gap size was significant ($F_{6,42} = 12.89$, $p < 0.05$). However, the main effect of conditioning/injection was not significant ($F_{1,7} = 0.211$, $p > 0.05$), indicating the blocking effect of 0.5 mg/kg MPEP injection on AFC. The startle responses, when the gap size was zero, were not affected by the treatment ($F_{1,7} = 0.955$; $p > 0.05$).

For the group of AFC/5 mg/kg MPEP injection (Fig. 5, low panels), following combination of AFC and 5 mg/kg MPEP injection, PPI was markedly reduced. Since a 7 by 2 two-way within-group ANOVA indicates that the interaction between gap size and treatment was significant ($F_{6,42} = 6.15$; $p < 0.05$), separate ANOVAs across various gap sizes and those
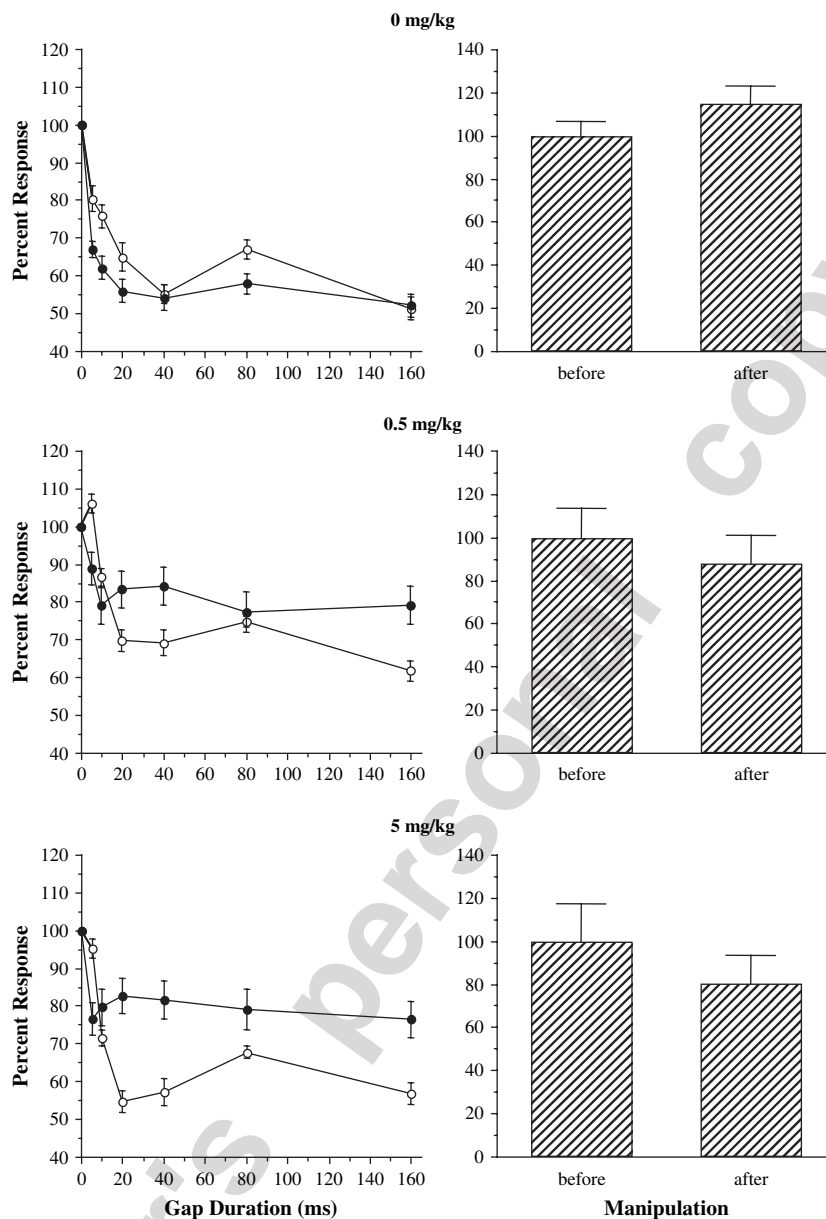
Fig. 5. Left panels: Mean percent startle responses as a function of the duration of the prepulse gap for the group of AFC/saline injection (up panel), the group of AFC/0.5 mg/kg MPEP injection (middle panel), and the group of AFC/5 mg/kg MPEP injection (low panel), respectively. Open circles, before gap-footshock pairing; filled circles, after gap-footshock pairing. Prepulse inhibition was significantly enhanced in the group of AFC/saline injection ($F_{1,10} = 5.240$, $p < 0.05$), unchanged in the group of AFC/0.5 mg/kg MPEP injection ($F_{1,7} = 0.211$, $p > 0.05$). For the group of AFC/5 mg/kg MPEP injection, prepulse inhibition was unchanged when the gap size was 5 ms ($F_{1,7} = 2.384$, $p > 0.05$), 10 ms ($F_{1,7} = 1.117$, $p > 0.05$), or 80 ms ($F_{1,7} = 1.010$, $p > 0.05$), but was significantly reduced when the gap size was 20 ms ($F_{1,7} = 10.233$, $p < 0.05$), 40 ms ($F_{1,7} = 16.909$, $p < 0.05$), or 160 ms ($F_{1,7} = 9.177$, $p < 0.05$). Right panels: Startle magnitudes under zero-gap condition before and after AFC/injection treatments. For each of the three groups, the startle responses, when the gap size was zero, were not significantly affected by the gap-footshock pairing and injection treatment (0 mg/kg group: $F_{1,10} = 3.386$, $p > 0.05$; 0.5 mg/kg group: $F_{1,7} = 0.955$, $p > 0.05$; 5 mg/kg group: $F_{1,7} = 2.667$, $p > 0.05$). Error bars indicate the standard error of the mean.

across treatment conditions were applied. When the gap size was 5, 10, or 80 ms, the AFC/injection treatment effect was not significant (5 ms: $F_{1,7} = 2.384$, $p > 0.05$; 10 ms: $F_{1,7} = 1.117$, $p > 0.05$; 80 ms: $F_{1,7} = 1.010$, $p > 0.05$). However, the treatment effect became significant when the gap size was 20 ms ($F_{1,7} = 10.233$, $p < 0.05$), 40 ms ($F_{1,7} = 16.907$, $p < 0.05$), or 160 ms ($F_{1,7} = 9.177$, $p < 0.05$). Thus MPEP injection at the dose of 5 mg/kg reversed the AFC effect when the gap prepulse was at some large sizes. On the other hand, the gap-size effect

before the AFC/injection treatment was significant ($F_{6,42} = 20.190$, $p < 0.01$). Pairwise comparisons show that the startle amplitude under zero gap-size condition was significantly different from those under all the other gap-size conditions except the one under the 5-ms gap-size condition. Thus before the AFC/injection treatment, this rat group exhibited marked PPI. However, the gap-size effect after the treatment was not significant ($F_{6,42} = 1.928$, $p > 0.05$), indicating a blocking effect of MPEP injection at the dose of 5 mg/kg on

AFC-induced enhancement of PPI. The startle responses, when the gap size was zero, were not affected by the treatment ($F_{1,7} = 2.667$; $p > 0.05$).

To further evaluate the effect of combination of AFC and MPEP injection on baseline startle, a one-way between-group ANOVA was conducted to compare the differences in the percent increase of baseline startle between the 3 groups. The calculation of percent increase of baseline startle was made using the following equation:

Percent increase $= 100\%$

$\times$ (post-treatment amplitude$-$ pre-treatment amplitude)/

pre-treatment amplitude

The results of the statistical analysis shows that the effect of combination of AFC and MPEP injection on baseline startle was not significant ($F_{2,24} = 0.888$, $p > 0.05$).

3.4. Effects of MPEP injection on prepulse inhibition

The effects of MPEP injection on gap-induced PPI of the acoustic startle reflex are shown in Fig. 6. For the group of 5 mg/kg MPEP injection only, a 7 by 2 two-way within-group ANOVA shows that the interaction between gap size and treatment was not significant ($F_{6,36} = 0.860$, $p > 0.05$), and the main effect of MPEP injection was not significant ($F_{1,6} = 0.000$, $p > 0.05$). The main effect of gap size was significant ($F_{6,36} = 11.865$, $p < 0.05$). The startle responses, when the gap size was zero, were not affected by the treatment ($F_{1,6} = 0.009$, $p > 0.05$).

4. Discussion

4.1. Gap induced prepulse inhibition

The temporal resolution of the auditory system, that is, the ability to discriminate rapid changes in the envelope of a sound, is important for processing the sound. A common way of investigating temporal resolution in both humans and animals is the measurement of the threshold of detecting a gap embedded in an otherwise continuous sound. The gap detection ability is determined in part by the rate of decay of neural activity during the gap and in part by sensitivity to the signal increment at the end of the gap (Plomp, 1964). Thus, compared to the detection of a sound burst, the detection of a gap involves more perceptual and/or cognitive components.

The gap has been successfully used as a prepulse in the PPI paradigm (Leitner and Girten, 1997; Barsz et al., 1998, 2002; Ison et al., 1998, 2002; Ison and Bowen, 2000). Unlike a sound-burst prepulse whose salience depends the sound level of the prepulse (Li et al., 1998), a gap prepulse can inhibit the startle reflex with different extents by varying the gap size without changing the sound level of the markers (or called carriers, the sounds before and after the gap). This feature of a gap prepulse is important for studying the dynamic function of prepulse without substantially changing the excitatory status of the auditory system. In the study by Ison and Bowen (2000), with the increase of the size of a gap embedded in noise markers from 0 to 10 ms, the inhibitory effect of the gap prepulse on the acoustic startle reflex in rats increased monotonically. However, it should be noted that gap-detection thresholds are dependent on several factors, such as the frequency spectrum and intensity of the markers, the dynamic onset and offset of the gap, and the room acoustics. Variations in parameters of these factors may obscure direct comparisons of results obtained from different laboratories.

The results of the present study confirm previous reports that a gap embedded in otherwise continuous background noise sounds can be used as a prepulse stimulus to inhibit the startle reflex (Leitner and Girten, 1997; Barsz et al., 1998, 2002; Ison et al., 1998, 2002; Ison and Bowen, 2000). The present results also show that PPI is largely determined by the gap size: with the increase of the gap size from 0 to 40 ms, the inhibitory effect of the gap on the acoustic startle reflex increases monotonically, indicating a dynamic range that is larger than that reported by Ison and Bowen (2000). Since the inhibitory effect is still significant when the gap
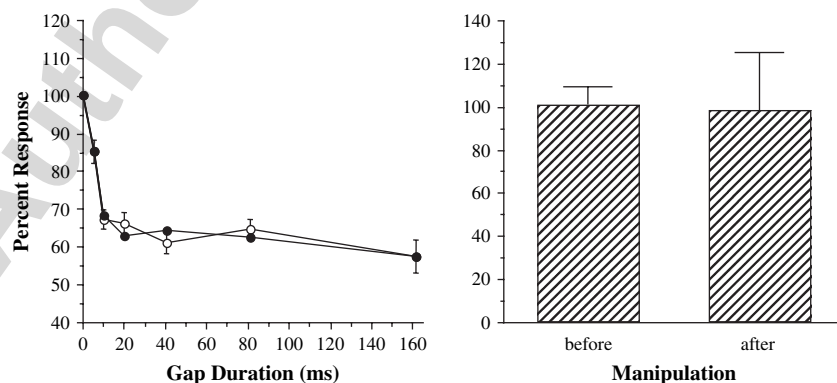

Fig. 6. Left panels: Mean percent startle responses as a function of the duration of the prepulse gap for the group of 5 mg/kg MPEP injection only. Open circles, before injection; filled circles, after injection. The gap-size effect was significant ($F_{6,36} = 11.865$, $p < 0.05$), but the MPEP-injection effect was not significant ($F_{1,6} = 0.000$, $p > 0.05$). Right panels: Startle magnitudes under zero-gap condition before and after MPEP injection. The startle responses, when the gap size was zero, were not affected by the injection ($F_{1,6} = 0.009$, $p > 0.05$). Error bars indicate the standard error of the mean.

size is as small as 5 ms, the results of the present study also suggest that for young rats the gap detection threshold can be less than 5 ms. Hence, the PPI paradigm used in the present study is useful for studying both auditory perception and sensorimotor gating.

4.2. Emotional learning enhances prepulse inhibition

In the present study, following temporally combining the 100-ms gap with footshock in a precise manner, the gap became conditioned, and gap-induced PPI was significantly enhanced. Thus when the gap becomes a signal informing aversive events, it elicits larger sensorimotor gating effects, compared to when it has not been conditioned. Previous studies suggest that the processing of the prepulse stimulus is highly correlated to the degree of PPI (Filion and Ciranni, 1994; Mussat-Whitlow and Blumenthal, 1997; Norris and Blumenthal, 1995, 1996; Perlstein et al., 1989, 1993). Thus the increase in PPI, following the prepulse stimulus being conditioned, indicates that deeper central processing is induced by AFC. It has been well documented that attention can enhance PPI (Dawson et al., 2000; Filion and Ciranni, 1994; Filion and Poje, 2003; Filion et al., 1993; Jennings et al., 1996; Schell et al., 1995, 2000; Thornea et al., 2005). One of the possible reasons of PPI enhancement is that conditioning the prepulse stimulus facilitates rats' attention to the prepulse stimulus.

Results of the functional magnetic resonance (fMRI) study by Hazlett et al. (2001) has shown that in the PPI testing paradigm, greater blood-oxygen-level-dependent (BOLD) responses occurred in the attention-related anterior and mediodorsal thalamic nuclei when subjects listened to attended prepulse tones than when they listened to ignored prepulse tones, and startling stimulus alone did not elicit such responses. However, large BOLD responses in the transitional medial cortex (Brodmann Area 32), which is involved in emotional processing of noxious stimuli, were elicited by the startling stimulus alone, but greatly inhibited by the attended prepulse sounds. In the future, the animal model established by the present study will be used for studying the neural mechanisms underlying fear-conditioning modulation of attention, and those underlying attentional modulation of PPI.

4.3. The effect of blocking the group I metabotropic glutamate receptors subtype 5

mGluR5 is critical for formation of AFC (Schulz et al., 2001; Fendt and Schmid, 2002; Lee et al., 2002; Rodrigues et al., 2002). In the present study, systemic injection of MPEP 30 min before AFC abolished the AFC effect, suggesting that mGluR5 is involved in the central processing that conditions the prepulse stimulus. It has been reported that fear conditioning induces an increase in expression of mGluR5 receptor protein (Riedel et al., 2000), and mGluR5 receptors have both structural and functional connections with *N*-methyl-D-aspartate receptors (NMDARs) (for a recent review see Simonyi et al., 2005). Increased expression of mGluR5 receptor protein may cause both an upregulation of NMDAR

functions and an increased reciprocal dependence between mGluR5s and NMDARs. It has been confirmed that blockade of NMDARs disrupts PPI (for a review see Geyer et al., 2001) and NMDARs in the amygdala are particularly responsible for the PPI disruption induced by NMDAR antagonists (Bakshi et al., 1999). In the future, it would be important to investigate whether the blocking effect of MPEP on AFC-induced PPI enhancement is mediated via NMDARs.

Consistent with results reported by previous studies (Henry et al., 2002; Kinney et al., 2003; Schulz et al., 2001; Spooren et al., 2000), the results of the present study also show that injection of MPEP has no effect on both baseline startle and PPI. It should be noted that MPEP has a potent anxiolytic effect (for a review see Spooren and Gasparini, 2004), and MPEP-induced reduction of fear and/or anxious responses may affect AFC. Thus there might be interactions between the effect of MPEP on emotion-related central process and the effect of MPEP on cognition-related central process. Several studies, however, have suggested that the two types of effects are not necessarily correlated. As reported by Ballard et al. (2005), the oral doses of MPEP (3—30 mg/kg p.o), which significantly induced a robust anxiolytic-like effect in rats, did not impair either working memory in the delay-match-to-position task or spatial learning in the Morris water maze. In addition, the acquisition and expression of conditioned place preference (CPP) induced by morphine (10 mg/kg) could be inhibited by a high dose of MPEP (30 mg/kg), which, however, had no effect on spatial learning and memory in the elevated plus maze (Popik and Wrobel, 2002). Moreover, systemic injection of MPEP (3 mg/kg, i.p.) (Schulz et al., 2001) or local administration of MPEP into the amygdala (Rodrigues et al., 2002) impaired the acquisition, but not expression, of AFC. Rodrigues et al. (2002) suggested that the failure of MPEP to influence the expression of fear memories rules out both the mGluR5 function in the retrieval of fear memories and the non-specific effects of MPEP on sensory processing at the time of training. Also as suggested by Schulz et al. (2001), since the sensitizing effect of footshock on the startle reflex is not blocked by MPEP, and the immediate motor response to the footshock (jumping and flinching in the test cage) are similar in vehicle- and in MPEP-treated rats, MPEP causes only a specific learning deficit that cannot be attributed to possible analgetic or sedative effects. Schulz et al. (2001) further argued that because both the acquisition and expression of AFC are impaired at the high dose of MPEP (30 mg/kg, oral administration), MPEP exerts anxiolytic properties only at high doses. Supporting Schulz et al.'s argument, results of the study by Brodkin et al. (2002) have indicated that intraperitoneal injection of MPEP one day after fear conditioning and 30 min before memory testing significantly reduced conditioned fear potentiation of startle if the injection dose was 10 or 30 mg/kg but not 3 mg/kg. However, intraperitoneal injection of a novel mGlu5 receptor antagonist, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP), after fear conditioning significantly reduced conditioned fear potentiation of startle at lower doses (1, 2.5, 3, or 5 mg/kg) (Busse et al., 2004; Pietraszek et al., 2005). As summarized in a recent

review article by Simonyi et al. (2005), it has become evident that mGluR5s play a critical role in fear conditioning, hippocampal-dependent spatial learning, avoidance learning, and conditioned taste aversion. Obviously, the effect of MPEP on fear/anxiety and that on learning depend on task type, route of administration, and dosage. In addition, as mentioned by Simonyi et al. (2005), considerable species- and strain-specific differences also exist. The association between the function of mGluR5 in mediating anxiety and that in mediating AFC still needs further investigation, particularly as suggested by Ballard et al. (2005), it would be important to determine the effect of systemic administration of MPEP on amygdaloid LTPs in vivo.

In summary, the present study, for the first time, demonstrated that following the prepulse stimulus, an energetic gap embedded in otherwise continuous broadband noise, was precisely combined with footshock and then became a signal informing the aversive event, PPI was significantly enhanced. Moreover, the AFC-induced PPI enhancement was reduced or even reversed by systemic administration of the mGluR5 antagonist, MPEP. Thus the present study established a model of AFC-induced modulation of gap-elicited PPI. This behavioral model would be useful for studying neural mechanisms underlying auditory temporal processing, sensorimotor gating, attention, AFC, and the interaction between emotional learning and sensorimotor gating. Specifically, this model will be used for investigating the functional interactions between mGluR5s and NMDARs.

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