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# Auditory Fear Conditioning Modulates Prepulse Inhibition in Socially Reared Rats and Isolation-Reared Rats

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Prepulse inhibition (PPI) is the reduction of the startle reflex when the startling stimulus is shortly preceded by a non-startling stimulus. Previous studies have shown that PPI in rats can be enhanced by auditory fear conditioning (AFC) but weakened by isolation rearing. This study investigated whether isolation rearing affects the effect of AFC on PPI. The results show that PPI was lower in isolation-reared rats than that in socially reared rats, and it was markedly enhanced by AFC in socially reared rats. However, the AFC-induced PPI enhancement in isolation-reared rats was much lower than that in socially reared rats. Moreover, the AFC-induced PPI enhancement was blocked by intraperitoneal injection (1 mg/kg) of the selective antagonist of metabotropic glutamate receptor subtype 5 (mGluR5), 2-methyl-6-(phenylethynyl)-pyridine (MPEP), 30 minutes before AFC. The baseline startle was also enhanced by isolation rearing. Thus, isolation rearing impairs not only PPI but also the AFC-induced PPI enhancement, which depends on mGluR5 activity. This study advances the animal model for investigating both neural bases and cognitive features of schizophrenia.

*Keywords:* auditory fear conditioning, metabotropic glutamate receptor subtype 5, prepulse inhibition, social isolation, 2-methyl-6-(phenylethynyl)-pyridine

The neurodevelopmental hypothesis of schizophrenia emphasizes that certain early-life environmental factors have substantial influences upon the processes of brain maturation and cause anatomical and functional abnormalities in the central nervous system (Ellenbroek & Cools, 1998; Marenco & Weinberger, 2000; Meyer, Feldon, Schedlowskib, & Yee, 2005; McGrath, Feron, Burne, Mackay-Sim, & Eyles, 2003; Rehn & Rees, 2005; Weinberger, 1996). Accordingly, several animal models involving early-life manipulations are proposed. One of the early-life manipulations is isolation rearing after weaning (21 days after birth in rats) (for a review, see Weiss & Feldon, 2001). Isolation rearing results in substantial changes in both neural structures/neurotransmissions (Dalley, Theobald, Pereira, Li, & Robbins, 2002; Day-Wilson, Jones, Southam, Cilia, & Totterdell, 2006; Harte et al., 2004; Heidbreder et al., 2001, 2000; Jones, Hernandez, & Kendall, 1992; Jones, Marsden, & Robbins, 1991; Lapiz, Mateo, Parker, & Marsden, 2000; Muchimapura, Mason, & Marsden, 2003; Preece, Dalley, & Theobald, 2004; Whitaker-Azmitia, Zhou, Hobin, & Borella, 2000) and behavior/cognition (Arakawa, 2005; Geyer, Wilkinson, Humby, & Robbins, 1993; Jones et al., 1991; Li, Wu, & Li, 2007; Paulus, Bakshi, & Geyer, 1998; Reboucas & Schmidek, 1997; Varty, Paulus, Braff, & Geyer, 2000; Weiss, Domeney, Moreau, Russig, & Feldon, 2001; Wilkinson et al., 1994).

It has been reported that schizophrenia patients usually suffer from impaired sensorimotor gating; namely, they have difficulty suppressing irrelevant sensory stimuli to ensure useful information processing (for reviews, see Braff, Geyer, & Swerdlow, 2001; Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001; Swerdlow, Geyer, & Braff, 2001; van den Buuse, Garner, Gogos, & Kusljic, 2005; Weiss & Feldon, 2001). A common operational model of sensorimotor gating is prepulse inhibition (PPI) of the startle reflex. The startle reflex is the strongest whole-body reflective response to intense sensory stimuli (Landis & Hunt, 1939), and PPI is the reduction of the startle reflex to an intense startling stimulus when this startling stimulus is shortly preceded by a weaker sensory stimulus (prepulse) (Graham, 1975; Hoffman & Ison, 1980; Ison & Hoffman, 1983; Li & Yue, 2002). PPI deficits in schizophrenia patients and schizotypal personality disordered subjects have been well documented (Braff, Grillon, & Geyer, 1992; Braff et al., 1978, Braff, Swerdlow, & Geyer, 1999; Cadenhead, Geyer, & Braff, 1993, Cadenhead, Swerdlow, Shafer, Diaz, & Braff, 2000; Grillon, Ameli, Charney, Krystal, & Braff, 1992; Karper et al., 1996; Kumari, Soni, Mathew, & Sharma, 2000; Kumari, Soni, & Sharma, 1999, Parwani et al., 2000; Perry & Braff, 1994; for a review, see Braff et al., 2001). Thus, PPI has been widely recognized as one of the potential cross-species models for studying schizophrenia (for reviews, see Braff & Geyer, 1990; Li & Shao, 2003). In rats, PPI deficits can be induced by early maternal separations and social isolation, and the deficits can be attenuated by both typical and atypical antipsychotics (Bakshi et al. 1998; Cilia, Hatcher, & Reavill, 2005; Cilia, Reavill, & Hagan, 2001; for reviews, see Braff et al., 2001; Geyer et al., 2001; Weiss & Feldon, 2001).

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Although PPI is an involuntary, preattentive process, it can be modulated by higher-order cognitive processes. In humans, PPI is enhanced when subjects selectively attend to the prepulse stimulus (Filion & Ciranni, 1994; Filion, Dawson, & Schell, 1993; Filion & Poje, 2003; Jennings, Schell, Filion, & Dawson, 1996; Schell, Dawson, Hazlett, & Filion, 1995, Schell, Wynn, Dawson, Sinaii, & Niebala, 2000; Thornea, Dawsona, & Schell, 2005). Also, eyeblink startle responses to a white-noise burst (103 dB, 50 ms in duration) are markedly inhibited by the presentation of a picture when the onset delay between the visual stimulus and the startling stimulus is 300 ms. Interestingly, the inhibition is affected by the content of the picture: either pleasant or unpleasant pictures causes larger inhibition than neutral pictures (Bradley et al., 2006). Moreover, results from Hazlett et al.'s (2001) functional magnetic resonance (fMRI) study 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.

Our recent studies confirmed that auditory fear conditioning (AFC) can enhance PPI in rats (Huang et al., 2007; Zou, Huang, Wu, & Li, 2007). Specifically, after the prepulse stimulus (either a sudden silent gap in background noise or a sudden change in correlation between the two sound sources) is precisely combined with footshock and the prepulse becomes conditioned, the strength of the prepulse stimulus in inhibiting the startle reflex is enhanced. These studies suggest that when the prepulse becomes biologically significant, the rat pays more attention to its occurrence. Thus, the attentional facilitation of PPI can be induced by AFC in rats.

The AFC-induced enhancement of PPI may involve the amygdala. The amygdala, especially the lateral nucleus (LA), plays a critical role in AFC (Fendt, 2001; Goosens & Maren, 2001; Maren, 1996; Romanski & LeDoux, 1992). In addition, the amygdala is involved in modulating PPI. For example, either large lesions of the amygdala or focal lesions of the basolateral nucleus of the amygdala significantly reduce PPI (Decker, Curzon, & Brioni, 1995; Fendt, Schwienbacher, & Koch, 2000; Stevenson & Gratton, 2004; Wan & Swerdlow, 1997; for reviews, see Li & Shao, 2003; Swerdlow et al., 2001). The LA receives auditory projections from the medial geniculate nucleus (MGN) (Doron & LeDoux, 1999; LeDoux, Farb, & Ruggiero, 1990; Romanski & LeDoux, 1993; Woodson, Farb, & LeDoux, 2000) and contributes to the development of neuronal plasticity in the MGN during AFC (Maren ,Yap, & Goosens, 2001; Poremba & Gabriel, 2001). The MGN has also been suggested to be an auditory structure that modulates PPI (Zhang, Engel, Ericson, & Svensson, 1999).

Social isolation results in significant neurotransmission abnormalities in the amygdala, including increased dopamine D-2 receptor density in the central nucleus of amygdala and reduced Fos-like immunoreactivity in the central and basolateral nuclei (Djouma, Card, Lodge, & Lawrence, 2006; Muchimapura, Fulford, Mason, & Marsden, 2002). Thus, it is important to know whether isolation rearing affects the AFC-induced enhancement of PPI. To our knowledge, this issue has not been addressed in the literature.

This study investigated whether social isolation affects the AFC modulation of PPI in rats. Moreover, because Group I metabotropic glutamate receptors subtype 5 (mGluR5) are essential for the formation of AFC (Fendt & Schmid, 2002; Lee, Lee, & Choi, 2002; Rodrigues, Bauer, Farb, Schafe, & LeDoux, 2002; Schulz et al., 2001; Zou et al., 2007) and contribute to glutamatergic dysfunction observed in patients of schizophrenia (Bach, Issac, & Slassi, 2007; Gupta et al., 2005; Pietraszek, Nagel, Gravius, Schaefer, & Danysz, 2007), this study also investigated the effect of administration of the selective antagonist of mGluR5, 2-methyl-6-(phenylethynyl)-pyridine (MPEP), on the AFC modulation of PPI in both socially reared rats and isolation-reared rats. The prepulse stimulus used in the present study was an energetic gap (a transient drop in sound level) embedded in otherwise continuous background noise sounds (Barsz et al., 2002; Ison, Agrawal, Pak, & Vaughn, 1998; Ison & Bowen, 2000; Leitner & Girten, 1997; Zou et al., 2007).

## Method

### *Subjects*

Forty-eight male Sprague-Dawley rats at the age of weaning (21 days old) were purchased from the Beijing Vital River Experimental Animals Technology Ltd. (Beijing, China). They were randomly assigned to two main groups: the socially reared group (24 rats) and the isolation-reared group (24 rats). Each of the two main groups was further randomly divided into three subgroups that received different manipulations: (1) AFC-control, (2) AFC/ Saline, and (2) AFC/ MPEP (for details see below).

For isolation-reared rats, each individual was housed in a single transparent plastic cage (48 × 30 × 18 cm). For socially reared rats, three individuals were housed in a cage with the same dimensions. Both isolated and socially reared rats were kept in the same room for eight weeks before testing. All rats had free access to food (Beijing Vital River Experimental Animals Technology Ltd., Beijing, China) and water. They were maintained under the condition of a constant temperature of 24 °C (± 2 °C) and a 12-hour light/dark cycle (lights on 07.00 h).

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 accordance with guidelines of the Beijing Laboratory Animal Center, the Canadian Council of Animal Care, and Policies on the Use of Animals and Humans in Neuroscience Research revised and approved by the Society for Neuroscience (January, 1995).

### *Apparatus*

The rat's whole-body startle reflex, which was induced by an intense 10-ms broadband noise burst (100 dB SPL) delivered by a loudspeaker 30 cm above the rat's head in a soundproof chamber, was measured by a custom-made electronic scale (the National Key Laboratory on Machine Perception, Peking University). 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) median cage: length = 139 mm, width = 52 mm, and height = 44 mm; (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 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 the rate of 16 kHz for 500 ms, beginning with the onset of the startling stimulus. Peak values during this interval were measured.

Two additional high-frequency loudspeakers, which were placed on the azimuthal plane in the frontal field with a 100° separation angle, were 52 cm away from the rat's head position (Figure 1). These two loudspeakers delivered continuous and independent broadband noise (55 dB SPL) as ambient background stimulation. They were also used to deliver gaps as prepulse stimuli. Sound levels were calibrated using a Brüel & Kjær (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.

### *Testing Procedures*

For the first three successive days, the rat was placed into the cage with its head extending out of the cage. The restrained rat was exposed only to the background acoustic stimulus (broadband noise in 55 dB SPL) for 20 minutes a day. The purpose of this pre-testing procedure was to allow the rat to become adapted to the restraining cage and the environment of the testing chamber.

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 background noise presented without the gap presentation. During testing, either a 100-ms gap or a 0-ms gap was presented from each loudspeaker without inter-loudspeaker delay. Fifty milliseconds after the end of the gap, the intense startling broadband-noise burst (100 dB SPL, 10 ms in duration) was presented by the top loudspeaker. About 30 s after the end of the gap, a new trial began. The inter-trial interval varied randomly between 25 s and 35 s. There were two different gap sizes, 0 and 100 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.

On the fifth day, all groups underwent AFC or AFC-control procedures. During AFC, the acoustic conditioned stimulus (CS) was the 100-ms gap delivered by the paired horizontal loudspeakers. Based on the previous studies (Sikes & Vogt, 1992; Villanueva et al. 1989; Zou et al., 2007), 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 unit (model PSIU6).

For the following four AFC 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) socially reared/AFC/saline, (2) socially reared/AFC/MPEP, (3) isolation-reared/AFC/saline, and (4) isolation-reared/AFC/MPEP. In each of these four groups, either saline or MPEP was injected 30 min before the procedure of AFC (see below).

For the following two AFC-control groups, the pairing of CS and US was in a randomly temporal manner: (1) isolation-reared/AFC-control, and (2) socially reared/AFC-control.

On the sixth day (24 hours after the manipulation), post-treatment PPI was measured using the same procedures.

### *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 the AFC in the following two groups: (1) socially reared/AFC/MPEP, and (2) isolation-reared/AFC/MPEP, with the dose of 1 mg/kg. For the other two AFC groups, only saline solution was administered intraperitoneally: (1) socially reared/AFC/saline, and (2) isolation-reared/AFC/saline. The injection volume for each animal was fixed at 1 mL.

### *Statistical Analyses*

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 (when the gap size = 0 ms). The following equation was used to calculate the percent response:

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

Thus, PPI equals to that 100% minus the percent response.

In addition, to compare the group differences in PPI gain induced by the manipulation on the fifth day, the following equation was used to calculate the PPI gain:

PPI gain = 100% × (PPI on the sixth day – PPI on the fourth day) / (PPI on the fourth day)

Analyses of variance (ANOVAs) and Scheffé tests were performed by using SPSS 11.5 software. The null-hypothesis rejection level was set at 0.05.

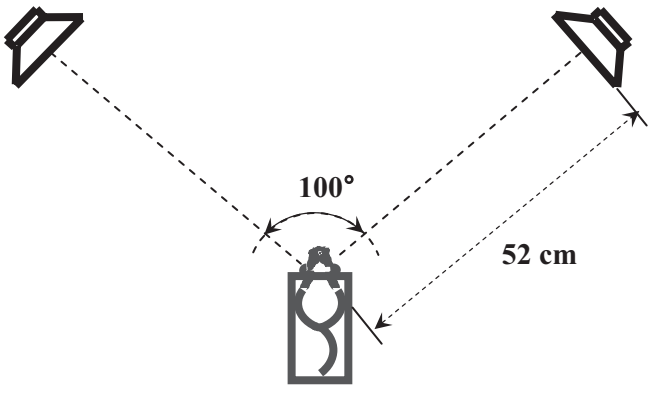


*Figure 1.* Diagram showing the spatial arrangement of the rat's body position and the two horizontal loudspeakers, which delivered the broadband-noise background and the prepulse gap.

## Results

### *Effects of Social Isolation on the Startle Reflex*

In the present study, a total of 24 rats were reared under the social condition and 24 other rats were reared under the isolation condition. The upper panel of Figure 2 shows the group-mean amplitude of the startle reflex (when the gap size = 0 ms) measured on the fourth day of testing (before manipulations) for both all the socially reared rats and all the isolation-reared rats. As shown in this panel, isolation-reared rats had the markedly larger startle amplitude than socially reared rats. A one-way between-group ANOVA shows that the difference in startle amplitude between socially reared rats and isolation-reared rats was significant, $F(1, 46) = 63.44$, $p < .001$.

As described in the "Methods" section, both socially reared rats and isolation-reared rats were further assigned into three groups with different manipulations conducted on the fifth day of testing: (1) AFC-control, (2) AFC/saline, and (3) AFC/MPEP. One-way ANOVAs show that on the fourth day of testing, there was no significant difference in startle amplitude between the three socially reared groups, $p = .985$, and there was no significant difference in startle amplitude between the three isolation-reared groups, $p = .837$ (also see Figure 3).

### *Effects of Social Isolation on PPI*

The lower panel of Figure 2 shows the group-mean normalized PPI measured on the fourth day of testing (before manipulations) for both all the socially reared rats and all the isolation-reared rats. As shown in this panel, isolation-reared rats had the markedly reduced PPI than socially reared rats. A one-way between-group ANOVA shows that the difference in PPI between socially reared rats and isolation-reared rats was significant, $F(1, 46) = 40.87$, $p < .001$.

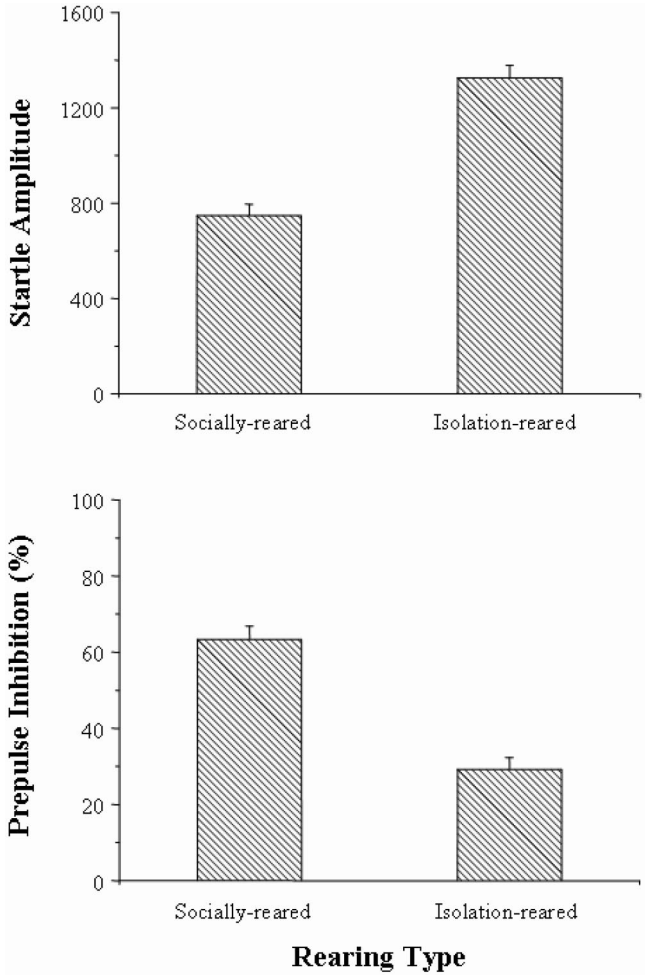


*Figure 2.* Upper panel: Comparison of group-mean amplitude of the startle reflex (measured on the fourth day of testing) between socially reared rats and isolation-reared rats before manipulations. The amplitude of startle reflex in isolation-reared rats was significantly higher than that in socially reared rats. Lower panel: Comparison of group-mean magnitude of normalized prepulse inhibition (PPI) between socially reared rats and isolation-reared rats before manipulations. The PPI magnitude in isolation-reared rats was significantly lower than that in socially reared rats. In this and following figures, error bars represent the standard errors of the mean.

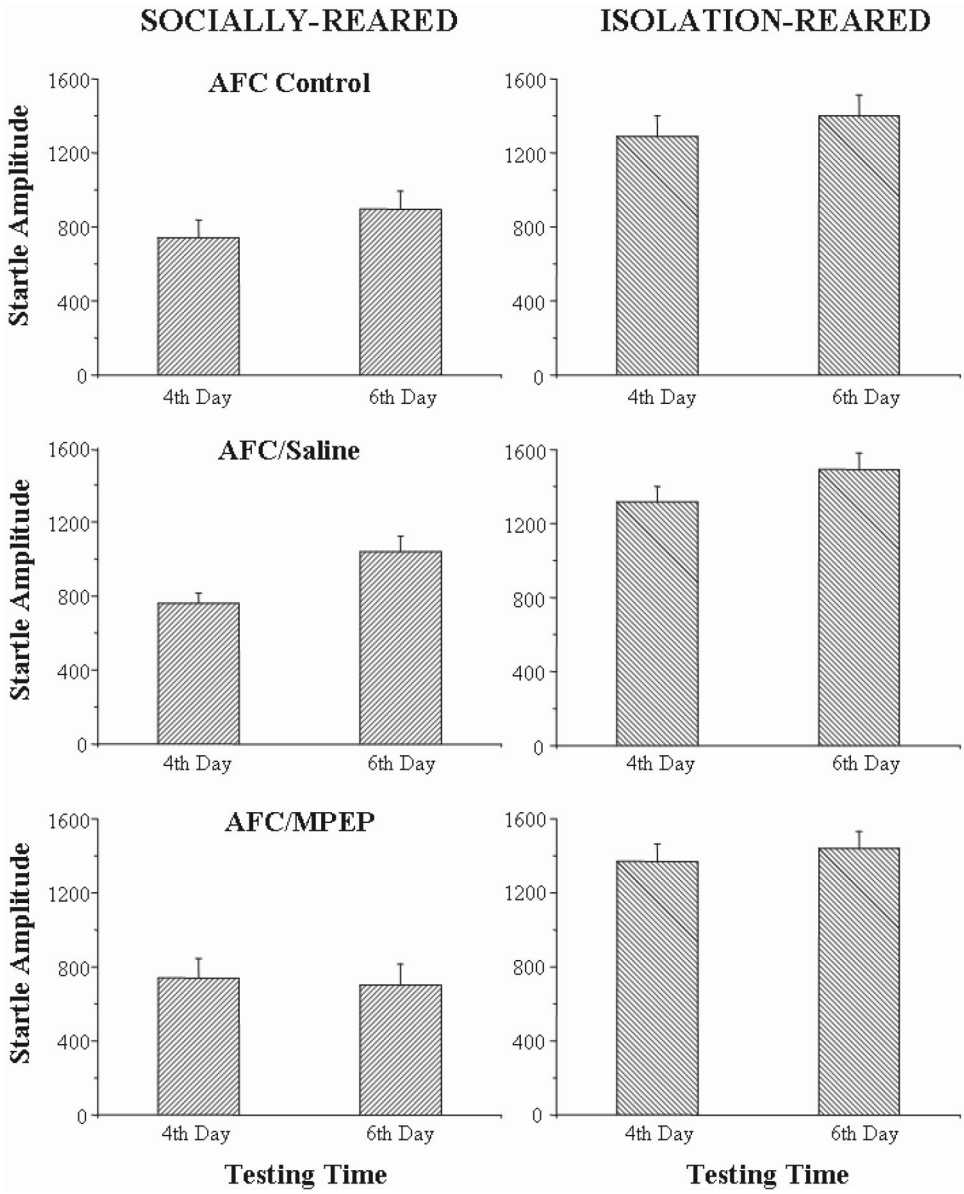


*Figure 3.* Comparison of group-mean amplitude of the startle reflex before and after different manipulations in socially reared rats and isolation-reared rats. Note that both the treatment auditory fear conditioning (AFC) control and the treatment AFC/saline significantly enhanced the startle reflex in both socially reared rats and isolation-reared rats. The treatment AFC/2-methyl-6-(phenylethynyl)-pyridine (MPEP) did not significantly affect the startle reflex in socially reared rats but significantly enhanced the startle reflex in isolation-reared rats.

Additional one-way ANOVAs show that on the fourth day of testing, there was no significant difference in PPI between the three socially reared groups (AFC-control, AFC/saline, AFC/MPEP) before manipulations, $p = .344$, and there was no significant difference between the three isolation-reared groups, $p = .110$ (also see Figure 4).

### *Comparison of AFC-Control Manipulation With AFC/Saline Manipulation in Affecting the Startle Reflex*

On the fifth day, a rat received one of the following three manipulations: AFC control, AFC/saline, and AFC/MPEP. On the sixth day, each rat was retested. Figure 3 show the group-mean startle amplitude for each of the six groups on the fourth day and that on the sixth day.

To estimate both the effect of the precise gap/footshock combination and the effect of the random gap/footshock combination on the startle reflex (when no prepulse gap was presented), comparisons of startle amplitude were made between rats with the manipulation AFC-control and those with the manipulation AFC/saline for both socially reared rats and isolation-reared rats before and after the manipulation. A 2 (rearing type: social, isolation) $\times$ 2 (manipulation type: AFC-control, AFC/saline) $\times$ 2 (testing time:

fourth day, sixth day) three-way ANOVA shows that for startle amplitude the interaction between the three factors was not significant, $p = .561$; the interaction between testing time and manipulation type was not significant, $p = .060$; the interaction between testing time and rearing type was not significant, $p = .147$; the interaction between rearing type and manipulation type was not significant, $p = .917$; the main effect of testing time was significant, $F(1, 28) = 52.51$, $p < .001$; the main effect of rearing type was significant, $F(1, 28) = 32.63$, $p < .001$; the main effect of manipulation type was not significant, $F(1, 28) = 0.62$, $p = .439$.

These analyses indicate that the temporal combination of the prepulse gap with footshock, regardless of the precise combination or the random combination, significantly enhanced startle in both socially reared rats (without MPEP injection) and isolation-reared rats (without MPEP injection). Also, the startle amplitude was higher in isolation-reared rats than in socially reared rats both before and after the manipulation.

### *Comparison of AFC-Control Manipulation With AFC/Saline Manipulation in Affecting PPI*

Figure 4 shows the group-mean normalized PPI for each of the six groups before and after the manipulation. To estimate the effects of the combination (precise or random) of gap presentation with footshock on PPI, comparisons were made between rats with the manipulation AFC-control and rats with the manipulation AFC/saline for both socially reared rats and isolation-reared rats before and after the manipulation.

A 2 (rearing type: social, isolation) × 2 (manipulation type: AFC-control, AFC/saline) × 2 (testing time: fourth day, sixth day) three-way ANOVA shows that for normalized PPI the interaction between the three factors was significant, $F(1, 28) = 19.88$, $p < .001$; the interaction between testing time and manipulation type was significant, $F(1, 28) = 30.58$, $p < .001$; the interaction between testing time and rearing type was significant, $F(1, 28) = 8.01$, $p < .01$; the interaction between rearing type and manipulation type was not significant, $p = .855$.

To examine the effect of testing time and the effect of manipulation type on PPI for socially reared rats and for isolation-reared rats, separate two-way ANOVAs were conducted.

For socially reared rats, a 2 (manipulation type) × 2 (testing time) ANOVA shows that the interaction between the two factors was significant, $F(1, 14) = 60.13$, $p < .001$ (the significant level was adjusted to 0.05/2). Further one-way separate ANOVAs show that for rats with the manipulation AFC-control, the effect of testing time was not significant, $p = .645$, but for rats with the manipulation AFC/saline, the effect of testing time was significant, $F(1, 7) = 690.83$, $p < .001$ (the significant level was adjusted to 0.05/4). These analyses suggest that for socially reared rats, the manipulation AFC/saline, but not the manipulation AFC-control, significantly enhanced PPI (see Figure 4).

For isolation-reared rats, a 2 (manipulation type) × 2 (testing time) ANOVA shows that the interaction between the two factors was not significant, $p = .496$; the main effect of testing time was not significant, $p = .060$; the main effect of manipulation type was not significant, $p = .188$. These analyses suggest that for isolation-reared rats, neither the manipulation AFC-control nor the manipulation AFC/saline caused significant effects on PPI.

### *Comparison of AFC/Saline Manipulation With AFC/MPEP Manipulation in Affecting the Startle Reflex*

As shown in Figure 3, compared to the manipulation AFC/saline, the manipulation AFC/MPEP did not cause a marked change in the startle amplitude for either socially reared rats or isolation-reared rats. A 2 (rearing type: social, isolation) × 2 (drug type: saline, MPEP) × 2 (testing time: fourth day, sixth day) three-way ANOVA shows that for startle amplitude the interaction between the three factors was significant, $F(1, 28) = 4.51$, $p < .05$; the interaction between testing time and drug type was significant, $F(1, 28) = 17.72$, $p < .001$; the interaction between testing time and rearing type was not significant, $p = .928$; the interaction between rearing type and drug type was not significant, $p = .311$.

To examine the effect of testing time and the effect of manipulation type on startle amplitude for socially reared rats and for isolation-reared rats, separate two-way ANOVAs were conducted.

For socially reared rats, a 2 (drug type) × 2 (testing time) ANOVA shows that the interaction between the two factors was significant, $F(1, 14) = 18.34$, $p < .01$. Further one-way separate ANOVAs show that for rats with the MPEP injection, the effect of testing time was not significant, $p = .051$ (the significant level was adjusted to 0.05/4), but for rats with the manipulation AFC/saline, the effect of testing time was significant, $F(1, 7) = 14.73$, $p < .01$.

For isolation-reared rats, a 2 (drug type) × 2 (testing time) ANOVA shows that the interaction between the two factors was not significant, $p = .143$; the main effect of testing time was significant, $F(1, 14) = 13.16$, $p < .01$; the main effect of drug type was not significant, $p = .992$.

These analyses show that the startle enhancement induced by precise gap/footshock combination was abolished by MPEP injection in socially reared rats but not in isolation-reared rats.

### *Comparison of AFC/Saline Manipulation With AFC/MPEP Manipulation in Affecting PPI*

Figure 4 shows that compared to the manipulation AFC/saline, the manipulation AFC/MPEP caused little effect on PPI in both socially reared rats and isolation-reared rats. A 2 (rearing type) × 2 (drug type) × 2 (testing time) three-way ANOVA shows that for normalized PPI the interaction between the three factors was significant, $F(1, 28) = 14.38$, $p < .01$; the interaction between testing time and drug type was significant, $F(1, 28) = 38.63$, $p < .001$; the interaction between testing time and rearing type was significant, $F(1, 28) = 11.82$, $p < .01$; the interaction between rearing type and drug type was not significant, $p = .361$.

To examine the effect of testing time and the effect of manipulation type on PPI for socially reared rats and for isolation-reared rats, separate two-way ANOVAs were conducted.

For socially reared rats, a 2 (drug type) × 2 (testing time) two-way ANOVA shows that the interaction between the two factors was significant, $F(1, 14) = 31.41$, $p < .001$. Further one-way ANOVAs show that for rats that received MPEP injection, the effect of testing time was not significant, $p = .705$, but for rats that received saline injection, the effect of testing time was significant, $F(1, 7) = 690.83$, $p < .001$.

For isolation-reared rats, a 2 (drug type) × 2 (testing time) two-way ANOVA shows that the interaction between two factors

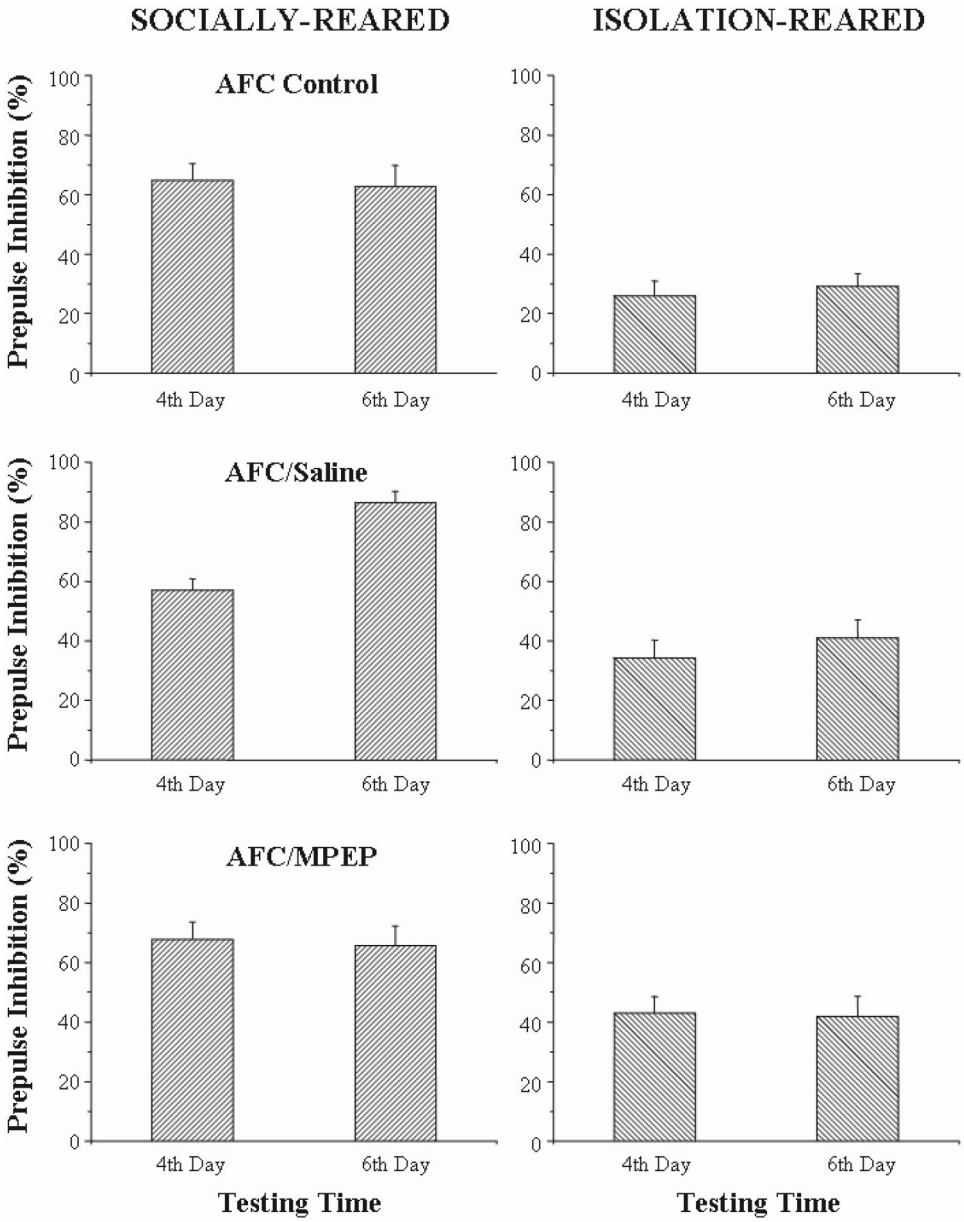


*Figure 4.* Comparison of group-mean magnitude of normalized prepulse inhibition (PPI) before and after different manipulations in socially reared rats and isolation-reared rats. Note that the treatment auditory fear conditioning (AFC)/saline, but neither the treatment AFC control nor the treatment AFC/2-methyl-6-(phenylethynyl)-pyridine (MPEP), caused a significantly enhancing effect on PPI in socially reared rats but a much weaker enhancing effect in isolation-reared rats.

was significant, $F(1, 14) = 7.24$, $p < .025$. Further one-way ANOVAs show that for rats that received MPEP injection, the effect of testing time was not significant, $p = .643$, but for rats that received saline injection, the effect of testing time was significant, $F(1, 7) = 13.33$, $p < .01$.

The analyses here suggest that the manipulation AFC/saline, but not the manipulation AFC/MPEP, significantly changed PPI in both socially reared rats and isolation-reared rats. Thus MPEP abolished the effect of enhancing PPI in both socially reared rats and isolation-reared rats.

### *Effects of Manipulations on Prepulse-Inhibition Gain*

Figure 5 and Table 1 show the PPI gain for each of the six rat groups. Obviously, only rats with the manipulation AFC/saline had markedly positive PPI gain. A 2 (rearing type) × 3 (manipulation type) two-way between-group ANOVA shows that for PPI gain the interaction between the two factors was significant, $F(2, 42) = 4.26$, $p < .05$.

For socially reared rats, a one-way ANOVA shows that the effect of manipulation type was significant, $F(2, 21) = 23.69$, $p <$

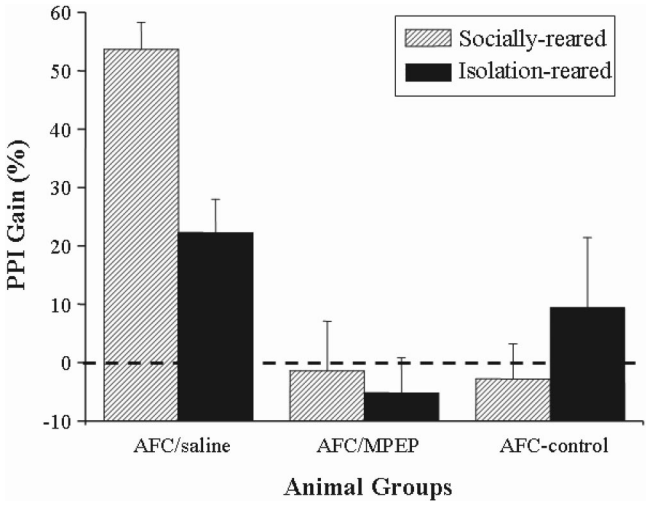


*Figure 5.* Comparisons of prepulse inhibition (PPI) gains between the six groups of rats. The broken line represents the PPI-gain value of zero. Note that in socially reared rats, the PPI gain for the auditory fear conditioning (AFC)/saline group was significantly higher than that for the AFC-control group and higher than that for the AFC/2-methyl-6-(phenylethynyl)-pyridine (MPEP) group. In isolation-reared rats, there was no significant difference in PPI between the three groups (AFC/saline, AFC/MPEP, AFC/control).

.001. Scheffé post tests show that rats with the manipulation AFC/saline had a significantly larger PPI gain than those with the manipulation AFC/MPEP and those with the manipulation AFC control, while rats with the manipulation AFC/MPEP had no significant difference in PPI gain from those with the manipulation AFC-control.

For isolation-reared rats, a one-way ANOVA shows that the effect of manipulation type was not significant, $p = .092$.

## Discussion

### *Isolation Rearing Reduces PPI*

Isolation rearing induces various schizophrenic-like cognitive/behavioral abnormalities in rats, including spontaneous hyperactivity in open field environments, impaired visual recognition memory, impaired reversal learning, and reduced PPI (Arakawa, 2005; Bianchi et al., 2006; Cilia et al., 2005, 2001; Geyer et al., 1993; Jones et al., 1991; Li et al., 2007; Paulus et al., 1998; Rebouças & Schmidek, 1997; Swerdlow et al., 2001; van den Buuse et al., 2005; van den Buuse, Garner, & Koch, 2003; Varty & Geyer, 1998; Varty et al., 2000; Weiss et al., 2001; Wilkinson et al., 1994). The present study, for the first time to our knowledge, demonstrates the deficits of gap-induced PPI in isolation-reared rats. Thus, the results of the present study are highly consistent with previous studies (for a review, see Weiss and Feldon, 2001), and support the view that reduced PPI in isolation-reared rats can be used for studying the neurodevelopmental roots of schizophrenia.

Table 1
*PPI Gains (Mean ± SE)*

| | AFC-control | AFC/saline | AFC/MPEP |
|---|---|---|---|
| Socially reared | −2.83 (6.00) | 53.69 (4.61) | −1.39 (8.61) |
| Isolation-reared | 9.46 (11.94) | 22.29 (5.76) | −5.18 (6.03) |

*Note*. AFC = auditory fear conditionig; MPEP = 2-methyl-6-(phenylethynyl)-pyridine.

Results of the present study also show that isolation-reared rats had stronger startle responses to the startling noise burst than socially reared rats. The enhanced startle in isolation-reared rats suggests that isolation-reared rats were more stressful than socially reared rats during testing. In the future, it would be necessary to investigate whether the isolation-induced startle enhancement is associated with certain altered neuronal responses to stress (Muchimapura et al., 2002).

### *Fear Conditioning Enhances PPI*

In the present study, compared to PPI before the manipulation AFC/saline-injection, PPI in socially reared rats after the temporally precise combination of footshock with the prepulse stimulus, but not the random combination, had a significantly larger magnitude. The results are consistent with our previous reports (Huang et al., 2007; Zou et al., 2007), showing that AFC of the prepulse stimulus enhances PPI. However, the PPI enhancement induced by AFC/saline-injection was much lower in isolation-reared rats.

Previous studies using human subjects suggest that either perceptual processing of or selectively paying attention to the prepulse stimulus enhances the magnitude of PPI (Dawson, Schell, Hazlett, Nuechterlein, & Filion, 2000; Filion & Ciranni, 1994; Filion et al. 1993; Filion & Poje, 2003; Jennings et al., 1996; Mussat-Whitlow & Blumenthal, 1997; Norris & Blumenthal, 1995, 1996; Perlstein, Fiorito, Simon, & Graham, 1989, Perlstein, Fiorito, Simon, & Graham, 1993; Schell et al., 1995, 2000; Thornea et al., 2005). Grillon and Davis (1997) also reported that even anticipation of aversive shock can enhance PPI. Thus, in the present study, conditioning the prepulse stimulus would facilitate

rats' attention towards the prepulse stimulus, enforce the processing of the prepulse stimulus, and enhance the magnitude of PPI.

### *Isolation Rearing Impairs Fear-Conditioning Enhancement of PPI*

The current study also shows that after the manipulation of AFC/saline, the enhancement of PPI in isolation-reared rats was much smaller than that in socially reared rats. Particularly, in isolation-reared rats the PPI gain induced by the manipulation of AFC/saline was not significantly different from that induced by either the manipulation AFC control or the manipulation AFC/MPEP. Thus, isolation rearing, not only reduces PPI, but also impairs the AFC-induced enhancement of PPI.

Isolation rearing results in a variety of attention deficiencies in rats, which are implicated in several neuropsychiatric syndromes, including schizophrenia (Dalley et al., 2002; Heidbreder et al., 2001, 2000; Preece et al., 2004). Isolation-induced attention deficits may explain why the AFC-induced enhancement of PPI in isolation-reared rats was smaller than that in socially reared rats. Supporting evidence to this hypothesis also comes from human studies in schizophrenic patients, who suffer from a variety of attentional disorders, such as hyper-distractibility and lateralized attention (Karper et al., 1996). In the studies by Kedzior and Martin-Iverson (2007), the eye-blink auditory startle reflex was recorded from the orbicularis oculi muscles while asking the participants to attend to or ignore either the 100 dB pulses or the 70 dB prepulse. The results show that schizophrenic patients showed a significant reduction in PPI relative to controls while attending to, but not while ignoring, the prepulse stimulus. Hazlett et al. (2003) have also reported similar deficits of attentional modulation of PPI in people with the schizotypal personality disorder. Thus, compared to normal people, schizophrenic patients have weakened PPI and/or reduced attention-induced enhancement of PPI.

In the present study, the duration of isolation rearing was 8 weeks. Considering that PPI and AFC do not have the same underlying mechanisms, one important issue that should be addressed in future studies is whether PPI and the AFC-induced PPI enhancement have the same or different vulnerabilities to isolation rearing. In other words, it is important to know whether a reduction of the isolation duration (or introducing controlled handling) decreases the PPI deficit and the PPI-enhancement deficit to the same or different degrees.

### *MPEP Blocks Fear-Conditioning Enhancement of PPI*

In the present study, systemic injection of 1mg/kg MPEP 30 min before AFC abolished the enhancing effect of AFC on PPI in socially reared rats, suggesting that mGluR5 is involved in the central processing of AFC. Indeed, it has been well documented that metabotropic glutamate receptors (mGluRs) play a key role in synaptic and behavioral plasticity, and mGluR5 is particularly critical for the formation of AFC (Fendt and Schmid, 2002; Lee et al., 2002; Rodrigues et al., 2002; Schulz et al., 2001; Zou et al., 2007). Fear conditioning can result in greater expression of the mGluR5 receptor protein (Riedel, Casabona, Platt, Macphail, & Nicoletti, 2000), and mGluR5 receptors have both structural and functional connections with N-methyl-D-aspartate receptors (NMDARs) (for a review see Simonyi, Schachtman, & Christoffersen, 2005). Increased expression of mGluR5 receptor protein may thus 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). Clearly, the functional interplay between mGluR5 and NMDARs in establishing the AFC-induced PPI enhancement is an important topic in future studies.

Isolation rearing results in various neurotransmission abnormalities, including serotonin, dopamine and glutamate (Dalley et al., 2002; Harte et al., 2004; Heidbreder et al., 2001; Jones et al., 1992, 1991; Muchimapura et al., 2003; Preece et al., 2004; Whitaker-Azmitia et al., 2000). More interestingly, Melendez, Gregory, Bardo, and Kalivas (2004) have recently reported an effect of the rearing condition on the expression of mGluR proteins in the prefrontal cortex (PFC). They found that the capacity of Group I mGluRs (mGluR1 and mGluR5) to elevate extracellular glutamate levels significantly decreased in the PFC of isolation-reared rats compared to rats reared in normal environmental conditions. As mentioned in the section of Introduction, mGluR5 not only are essential for the formation of AFC (Fendt & Schmid, 2002; Lee et al., 2002; Rodrigues et al., 2002; Schulz et al., 2001; Zou et al., 2007) but also contribute to glutamatergic dysfunction observed in patients of schizophrenia (Bach et al., 2007; Gupta et al., 2005; Pietraszek et al., 2007). Also, in rats, the selective mGluR5 antagonist, MPEP, potentiates the disruptions in radial maze tasks induced by phencyclidine (Campbell et al., 2004), and the other selective antagonist of mGluR5, 3-[( 2- methyl- 1,3- thiazol- 4- yl) ethynyl] pyridine, (MTEP), dose-dependently induces social isolation that is considered to reflect social deficits of negative schizophrenia symptoms (Koros, Rosenbrock, Birk, Weiss, & Sams-Dodd, 2007). Thus, the mechanisms associated with mGlu5 underlying isolation-induced PPI deficits and PPI-enhancement deficits need further investigations in the future. As mentioned above, differentiating the vulnerability to isolation rearing for PPI and that for the AFC-induced PPI enhancement is essential for this line of investigation.

In summary, the present study shows that a temporally precise combination with footshock with the prepulse stimulus (100-ms gap) significantly enhanced PPI in socially reared rats. However, the PPI enhancement in isolation-reared rats was much smaller. Moreover, the AFC-induced PPI enhancement was abolished by systemic administration of the mGluR5 antagonist, MPEP. Thus this study advances the animal model for investigating both neural bases and cognitive features of schizophrenia.

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