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Research paper

The influence of the perceptual or fear learning on rats' prepulse inhibition induced by changes in the correlation between two spatially separated noise sounds

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Abstract

Perceptually grouping a sound source with its reflections and separating them from irrelevant background noise sounds need computation of sound correlations and are critical for identifying and localizing the sound source in a complex acoustic environment. Using the prepulse inhibition of the acoustic startle reflex (ASR) as a measure, the present study investigated whether rats are able to detect correlation changes between sounds from different spatial locations. The results show that the rat's ASR amplitude was suppressed when the startle-eliciting stimulus was preceded by either an uncorrelated noise fragment or an anti-phase noise fragment that was embedded in two identical (correlated) but spatially separated noises. Suppression of the ASR amplitude increased as the duration of the noise fragment increased from 5 ms to 40 ms. The suppressive effect was also progressively enhanced after rats underwent successive testing sessions. Moreover, an enhanced suppression of the ASR amplitude was observed after rats were exposed to footshock that was precisely paired with a 100-ms correlation-change fragment. The results indicate that rats are able to detect the correlation change between sounds from two separated spatial locations, and the detection can be facilitated by both perceptual learning and emotional learning.
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1. Introduction

In noisy, reverberant environments, listeners receive not only the sound waves that directly come from sound sources, but also numerous reflections of the sources from various directions. To identify and localize an individual sound source, the auditory system needs to not only differentiate the target sound from irrelevant sounds, but also to perceptually group the target sound with its reflections. Therefore, both computation of correlations between sounds and detection of changes in sound correlation are critical for processing acoustic signals in complex environments.

Human auditory system is very sensitive to subtle changes in the correlation between acoustic signals reaching the two ears. Human listeners can tell the difference between an exact copy (interaural correlation coefficient $= 1.0$) and a nearly exact copy (interaural correlation coefficient $\geqslant 0.96$) delivered to the other ear while the original signal is delivered to one of the ears (Gabriel and Colbum, 1981; Pollack and Trittipoe, 1959). Furthermore, human listeners can detect the occurrence of either a transient break in correlation (i.e., a transient drop of correlation coefficient from 1.00 to 0 and then return to 1.00) or a

transient phase reversal (i.e., a transient drop of correlation coefficient from 1.00 to −1.00 and then return to 1.00) between signals delivered to the two ears (Akeroyd and Summerfield, 1999; Boehnke and Hall, 2002). To understand humans' ability of detecting dynamic changes in sound correlation, animal physiological and behavioral studies are useful and even necessary. However, up to date, little has been known about whether laboratory animals (such as rats) are sensitive to changes in correlation between sounds delivered from different sources.

The acoustic startle reflex (ASR) is a type of whole-body reflective response to a sudden and intense sound (Landis and Hunt, 1939). The amplitude of the ASR can be reduced by a weak, non-startling sound presented shortly before the startling sound. This phenomenon has been called prepulse inhibition (PPI) (Braff and Geyer, 1990; Graham, 1975; Hoffman and Ison, 1980; Ison and Hoffman, 1983; Li and Yue, 2002; Li and Shao, 2003). Substantial evidence has indicated that the degree to which the ASR amplitude is reduced (i.e., the magnitude of PPI) can be used as a measure of the salience of the prepulse stimulus in both humans and rodents (Barsz et al., 1998, 2002; Carlson and Willott, 1996; Filion and Ciranni, 1994; Hoffman and Ison, 1980; Ison and Bowen, 2000; Leitner and Girten, 1997; Li et al., 1998; Mussat-Whitlow and Blumenthal, 1997; Norris and Blumenthal, 1996; Perlstein et al., 1993; Turner et al., 2006; Young and Fechter, 1983). As summarized in a recent paper by Turner et al. (2006), using the PPI paradigm to study animals' detection of an acoustic event (e.g., a silent gap) has several advantages, including: (1) no food or water deprivation, (2) no training demands, (3) short testing time, and especially (4) known neural circuits mediating PPI (for reviews see Fendt et al., 2001; Li and Yue, 2002). In the present study, the first purpose was to use the PPI paradigm to examine whether rats are able to detect transient changes of correlation between sounds.

PPI reflects a protection of early processing of the prepulse signal (Graham, 1975). The magnitude of PPI is determined by complicated interactions between target-signal processing, distracting-stimulus filtering, and top-down modulation. Although PPI occurs without conditioning of the prepulse stimulus, the circuits mediating/modulating PPI are not "hard wired". In humans, PPI can be enhanced by shifting attention to the prepulse (Bohmelt et al., 1999; Dawson et al., 1993; Filion and Ciranni, 1994; Filion and Poje, 2003; Filion et al., 1993; Heekeren et al., 2004; Jennings et al., 1996; Schell et al., 1995; Schell et al., 2000; Thornea et al., 2005), indicating that PPI contains a considerable capacity of plasticity. Up to date, there have not been sufficient studies of examining cognitive modulations of PPI in laboratory animals.

It has been well known that training improves sensory performances (Gibson and Gibson, 1955). For auditory perceptual tasks in humans, perceptual learning improves frequency discrimination (e.g., Demany, 1985), temporal-interval discrimination (e.g., Wright et al., 1997), and performances involving binaural processing, such as sound localization (Abel and Paik, 2004), interaural-cue discrimination (Wright and Fitzgerald, 2001), and reduction of lag suppression (Saberi and Perrott, 1990). For sound discrimination tasks in rats, passive exposure to the target sound can improve the discrimination of two amplitude-modulated tones with different modulation depths (Sakai and Kudoh, 2005). Thus it is of interest to know whether the effect of perceptual learning on sensory detection can be measured by the PPI paradigm in both humans and laboratory animals. The second purpose of this study was to use the PPI paradigm to investigate the effect of repeated presentations of the prepulse, which is a change in inter-sound correlation, on PPI in rats.

Our recent studies have shown that rats' PPI induced by an energetic gap embedded in otherwise continuous noise sounds can be enhanced by temporally pairing the gap with footshock (Zou et al., 2006). When a neutral prepulse sound is temporally combined with footshock, the prepulse sound becomes conditioned and signifies the aversive event, in other words, auditory fear conditioning (AFC) occurs. AFC makes the prepulse stimulus more salient, and consequently, enhances PPI. The third purpose of this study was to use the PPI paradigm to investigate whether rats' detection of changes of correlation between two spatially separated sounds can be enhanced by AFC.

In this study, we tested the hypothesis that rats are able to detect an uncorrelated (UC) noise fragment and/or an anti-phase (AP) noise fragment embedded in two identical (correlated) broadband noises, which are presented from two spatially separated locations in the azimuthal plane. We used the prepulse-reduced ASR as the measure of rats' ability of detecting the UC or AP fragment. We further investigated whether the rats' ability of detecting the correlation changes can be improved by perceptual learning and/or AFC.

2. Methods

2.1. Subjects

Forty young adult male albino Sprague–Dawley rats (weight between 250 g and 350 g), which were purchased from the Beijing Vital River Experimental Animals Technology, were used in this study. They were in excellent heath conditions and free of infection and/or obstruction in the external ears. They were housed individually in plastic cages and placed on a 12-h light/dark cycle, with food and water freely available. They were allowed 6 days to adapt to the housing environment before the testing. During the testing, animals were randomly divided into the following two main groups: (1) UC prepulse group (20 rats); (2) AP prepulse group (20 rats). All efforts were made to minimize animal suffering. The experiments were carried out in according with the Guidelines of the Beijing Laboratory Animal Center, and the Guidelines of the Canadian Council of Animal Care.

2.2. Apparatus and materials

The rat's whole-body startle reflex, which was elicited by an intense 10-ms broadband noise burst (100 dB SPL) delivered by a loudspeaker (PCxb 352, Blaupunkt, USA) 30 cm above the rat's head, was measured by an electrical scale in a soundproof chamber. The scale had a platform, on which a specially designed small metal-mesh restraint cage was placed. There were three different sizes of the cages for different body weights. The internal dimensions of the three sizes 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; (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 startle reflex. This voltage was amplified and passed through an analog/digital–digital/analog converter. Startle-induced electrical voltages were sampled at a frequency of 16 kHz for 500 ms, beginning with the onset of the startling stimulus.

Two additional loudspeakers of the same model (PCxb 352, Blaupunkt, USA), 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. Although the frequency response of the loudspeaker was in the range of 90 Hz to 20 kHz, the sampling rate of the playback system limited the spectrum of the acoustic signal to frequencies below 8 kHz. These two horizontal loudspeakers delivered identical (correlation coefficient = 1) broadband noise continuously as the background sound. There was no delay between the two loudspeakers.

A UC fragment (correlation coefficient = 0) or an AP fragment (correlation coefficient = −1) was inserted in the background sound as a prepulse stimulus, whose duration was 0 ms, 5 ms, 10 ms, 20 ms, 40 ms, or 80 ms. It should be noted that adding the noise fragment did not change the long-term spectrum and the sound level of the noise sound delivered from each of the loudspeakers. The intense startle-eliciting noise burst was presented by the top loudspeaker following the UC or AP fragment with the inter-stimulus interval (ISI) of 120 ms (Fig. 1).

White-noise signals were digitally generated by the Matlab 'randn' function and converted by a custom-developed sound playback system (the National Key Laboratory on Machine Perception, Peking University) with the 16-kHz sampling rate and 16-bit resolution. 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. The two horizontal loudspeakers continuously delivered correlated noise sounds (except when UC fragments or AP fragments occurred) without phase delay. The single-source sound level for each of the two horizontal loudspeakers was fixed at the level of 56 dB SPL. A Pentium IV microcomputer placed outside the soundproof
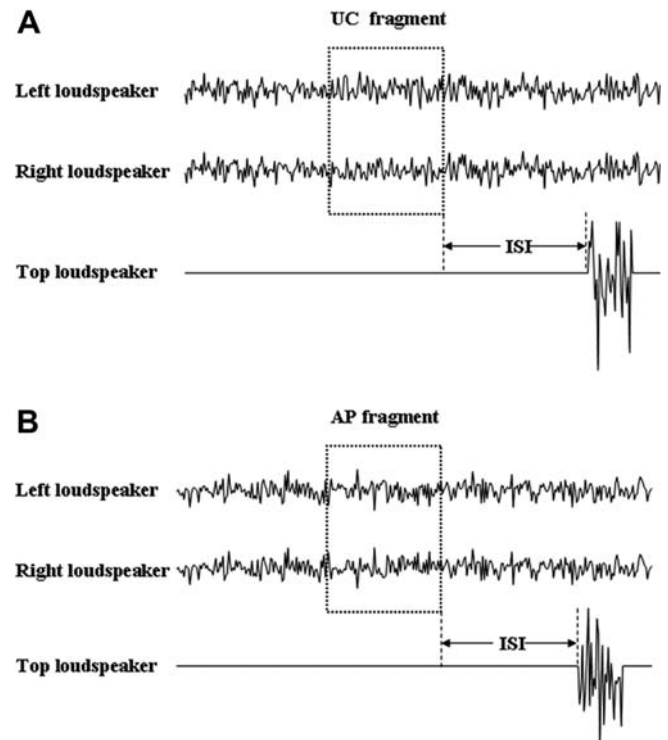

Fig. 1. Waveforms of the prepulse stimulus delivered from the left and the right horizontal loudspeakers and those of the startle-evoking stimuli delivered from the top loudspeaker. The duration of the correlation-change fragment was fixed at 5 ms, 10 ms, 20 ms, 40 ms, or 80 ms in each testing trial. The inter stimulus interval (ISI) between the fragment and the startle-evoking noise burst was fixed at 120 ms. Panel A: An uncorrelated (UC) noise (correlation coefficient = 0) was embedded in the two identical noises as a prepulse stimulus. Panel B: An anti-phase (AP) noise fragment (correlation coefficient = −1) was embedded in the two identical noises as a prepulse stimulus.

chamber was used to run experimental programs. The stimulus presentations and startle measurements were also controlled by the computer. Data were analyzed with the custom-developed program (the National Key Laboratory on Machine Perception, Peking University).

2.3. Procedures

2.3.1. Stage1: perceptual learning

On the seventh day of the arrival in the laboratory, the rat was placed into the cage with its head extending out of the cage. The restrained rat was first exposed to acoustic stimuli used for PPI testing (see below) for 30 min, and then a testing trial started. One hundred and twenty milliseconds after the end of the fragment (UC fragment or AP fragment), the intense startling noise burst was presented by the top loudspeaker. About 30 s later, a new trial began. The inter-trial interval randomly varied in the range of 25–35 s with the mean of 30 s.

Both the UC fragment and the AP fragment had six different durations, and each fragment was presented 15 times in one testing session. The order of presenting fragments of different durations was in a random fashion. Each rat was

tested just for one session (90 trials) per day. For an individual rat, the same testing procedure was repeated for at least four successive days, and stopped if stable PPI was obtained at each of the fragment durations for two successive days. The purpose of this procedure was to test whether rats' PPI would change with the repeated exposure to the testing conditions.

2.3.2. Stage 2: emotional learning (auditory fear conditioning)

Rats (17 from the UC-fragment group and 18 from the AP-fragment group) with stable startle responses in Stage 1 were used as the subjects in Stage 2 of testing. Rats' startle responses on the last day of Stage 1 were treated as pre-conditioning responses. These rats were further assigned into the following four subgroups: (1) UC-fragment/AFC (9 rats); (2) UC-fragment/AFC-control (8 rats); (3) AP-fragment/AFC (9 rats); (4) AP-fragment/AFC-control (9 rats).

In either AFC or AFC-control procedures, the conditioned stimulus (CS) was a 100-ms UC or AP fragment delivered by the horizontal loudspeakers. Based on the study by Sikes and Vogt (1992) and that by Villanueva et al. (1989), the unconditioned stimulus (US) was 6 mA footshock (duration = 3 ms), which was introduced by a Grass current stimulator (Grass Model S88K, Quincy, Massachusetts, USA). The electrical shock was delivered through two small pieces of platinum plates taped to one of the rat's hindpaws. For the two AFC groups, 10 precisely combined pairs of CS and US (footshock started 3 ms before the noise fragment ending, and co-terminated with the fragment) were presented with the repetition rate around 30 s. For the two AFC-control groups, the pairing of CS and US was in a randomly temporal manner. Timing of sound stimuli and footshock were controlled by the experimental program through computer.

One day after the CS–US pairing, the rat was tested again with the same procedure used in Stage 1.

2.4. 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 (fragment duration = 0 ms). The following equation was used to calculate the percent response:

Percent response = $100\% \times$ (amplitude to startling sound

with a prepulse/amplitude to startling sound alone)

To evaluate which fragment size could be detected by rats, we compared the amplitude of ASR when the fragment duration was 5 ms, 10 ms, 20 ms, 40 ms, or 80 ms with that when the fragment duration was 0 ms, using a one-way ANOVA. To test the effect of CS/US pairing, a 5 (prepulse duration) by 2 (before and after conditioning)

two-way within-participant ANOVA was used. The analyses were performed using SPSS 13.0 software. The null-hypothesis rejection level was set at 0.05.

3. Results

3.1. Stage 1: perceptual learning

During the Stage 1 of testing, each of the tested rats exhibited robust startle responses to the intense sound delivered from the top loudspeaker. However, three rats from the UC-fragment group and two rats from the AP-fragment group were ruled out, because of their unstable behavioral status in the recording cage. The remained rats adapted well to the restraining condition. In each of them, the intense startling noise burst reliably elicited whole-body startle responses, whose latencies of the primary peak components were about 15 ms after the onset of the startling noise burst. When a UC or AP fragment, particularly with a large duration, preceded the startle-evoking stimulus, the amplitude of rats' startle response was remarkably suppressed after the perceptual learning procedure.

3.1.1. UC-fragment group

For the UC-fragment group, on the first two days of perceptual learning, there was little inhibitory effect of the UC-fragment prepulse on the ASR. However, with the testing being continued, the inhibitory effect became marked (Fig. 2). A one-way ANOVA shows a significant effect of testing days on PPI for this group ($F_{5,30} = 5.73$, MSE=855.283, $p = 0.001$), indicating a perceptual learning effect on PPI. Further separate one-way ANOVAs show that the UC-fragment duration effect on PPI was not significant on the first two testing days (Day 1: $F_{5,84} = 0.219$, MSE=146.330, $p = 0.954$; Day 2: $F_{5,84} = 1.567$, MSE = 934.326, $p = 0.178$), marginally significant on the third testing day ($F_{5,84} = 2.057$, MSE=1081.980, $p = 0.076$), but highly significant on the fourth, fifth, and sixth testing days (Day 4: $F_{5,84} = 5.606$, MSE = 2438.848, $p < 0.001$; Day 5: $F_{5,84} = 7.195$, MSE = 3802.180, $p < 0.001$; Day 6: $F_{5,84} = 17.763$, MSE = 5030.867, $p < 0.001$).

Multiple comparisons indicate that on Days 1 and 2, compared to the baseline ASR (when the UC-fragment duration was 0 ms), the startle responses at all the UC fragment durations were not significant from the baseline ASR ($p > 0.05$). On Day 3, the startle responses only under the condition of the 80-ms fragment duration were significantly different from the baseline ASR. On Day 4, the startle responses under conditions with large UC-fragment durations (20 ms, 40 ms, and 80 ms) were significantly different from the baseline ASR. On Days 5 and 6, the startle responses under all the UC-fragment durations (5 ms, 10 ms, 20 ms, 40 ms, and 80 ms) were significantly different from the baseline ASRs. The results indicate a progressively increased sensitivity to the UC fragment with perceptual learning (Fig. 4, top panel).
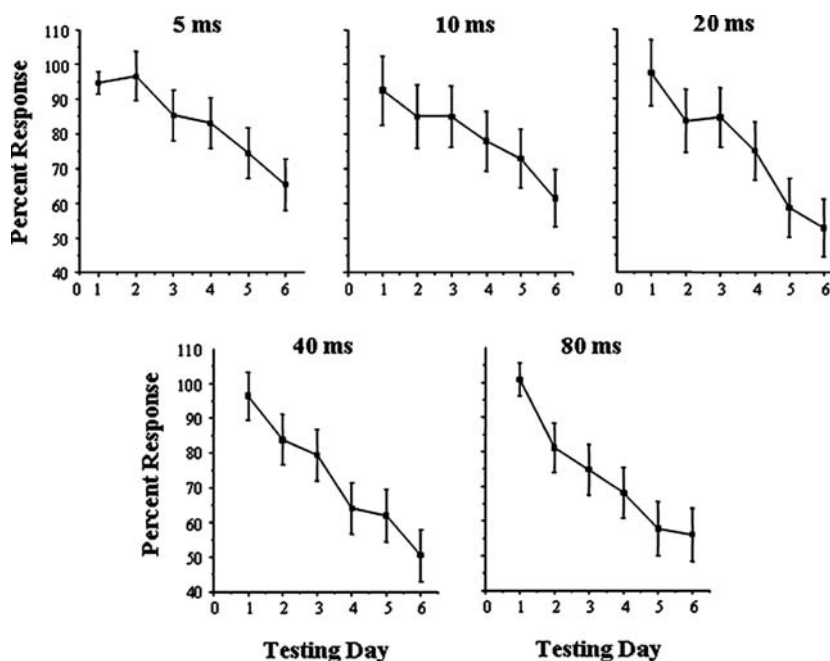
Fig. 2. Effects of the number of testing days during perceptual learning on the amplitude of the acoustic startle reflex (ASR) with the UC fragment of 5 ms, 10 ms, 20 ms, 40 ms, or 80 ms. The suppressive effect of the UC fragments on the amplitude of ASR increased as the number of testing days increased.

To examine whether the baseline startle response (when fragment duration = 0 ms) was stable during the course of perceptual learning in the UC-fragment group, a within-participant ANOVA was conducted. The results show that the fluctuation of baseline startle response was not significant over the six testing days, $F_{5,84} = 0.962$, MSE = 3,561,361.997, $p = 0.446$.

3.1.2. AP-fragment group

For the AP-fragment group, compared to the startle responses under the no-prepulse condition (when the AP fragment duration = 0 ms), startle responses were clearly inhibited by presenting the AP-fragment prepulse with the duration of 80 ms on the first day of perceptual learning (Fig. 3). However, for those fragments with small
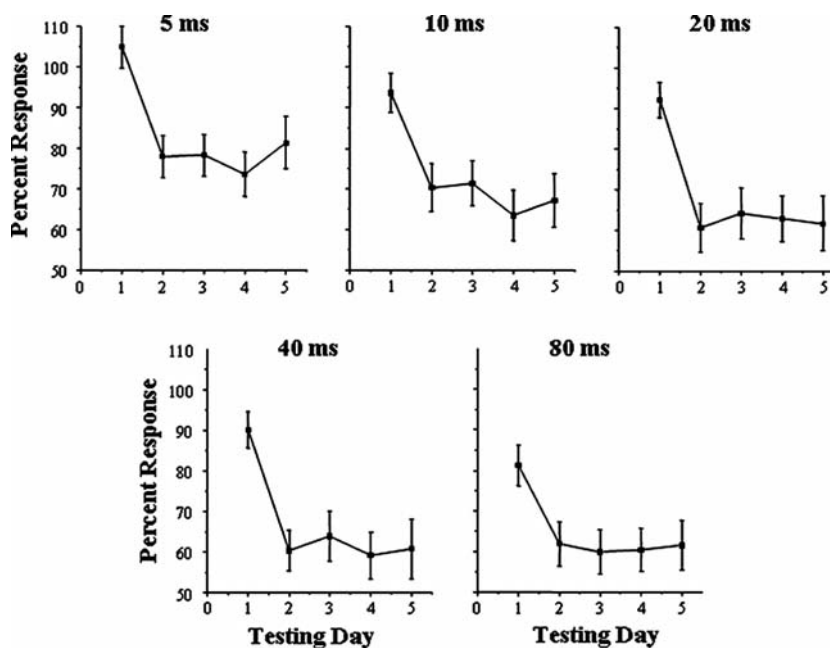

Fig. 3. Effects of the number of testing days during perceptual learning on the amplitude of the ASR with the AP fragment of 5 ms, 10 ms, 20 ms, 40, or 80 ms. The suppressive effect of the AP fragments on the amplitude of ASR increased from Day 1 to Day 2, but did not change as testing continued after Day 3.

durations, PPI was not apparent on the first day of perceptual learning. A one-way ANOVA for the performance on Day 1 indicates that there was a significant duration effect on PPI ($F_{5,96} = 3.511$, MSE = 1143.102, $p = 0.006$). Multiple comparisons confirm that the startle responses with 80-ms AP fragment was significantly different from that with 0-ms AP fragment (baseline ASR) ($p < 0.05$). Startle responses with the AP-fragment duration of 5 ms, 10 ms, 20 ms, or 40 ms were not significantly different from that with 0-ms AP fragment ($p > 0.05$).

On Day 2 of perceptual learning, PPI in the AP-fragment group markedly improved. Moreover, with the increase of number of testing days, PPI became stable (also see Fig. 4b). A one-way ANOVA shows that there is a significant effect of testing days on PPI for the AP-fragment group ($F_{5,25} = 2.892$, MSE = 586.647, $p = 0.043$), indicating a perceptual learning effect on PPI. Separate one-way ANOVAs show a significant effect of the AP-fragment duration on PPI on these testing days (Day 2: $F_{5,96} = 9.366$, MSE = 4043.555, $p < 0.001$; Day 3: $F_{5,96} = 7.977$, MSE = 3711.359, $p < 0.001$; Day 4: $F_{5,96} = 9.030$, MSE = 4137.234, $p < 0.001$; Day 5: $F_{5,96} = 6.666$, MSE = 4205.777, $p < 0.001$). Compared to the baseline startle (when the AP-fragment duration = 0 ms), the startle response at AP-fragment durations of 5 ms, 10 ms, 20 ms, 40 ms, and 80 ms were significantly smaller ($p < 0.05$) after Day 2, indicating that following perceptual learning rats could detect an AP fragment with the duration of at least 5 ms (Fig. 4b).

To examine whether the baseline startle response was stable during the course of perceptual learning in the AP-fragment group, a within-participant ANOVA was also conducted. The results show that the fluctuation of baseline startle response was not significant over the 5 testing days, $F_{4,78} = 0.219$, MSE = 2,602,311.062, $p = 0.927$.

3.2. Stage 2: emotional learning

As mentioned above, 17 rats in the UC-fragment group and 18 rats in the AP-fragment group were tested during the perceptual learning (Stage 1). Their startle responses on the last day of Stage 1 were treated as pre-AFC responses. In emotional learning tests (Stage 2), these rats were further assigned into the following four subgroups: (1) UC-fragment/AFC (9 rats); (2) UC-fragment/AFC-control (8 rats); (3) AP-fragment/AFC (9 rats); (4) AP-fragment/AFC-control (9 rats).

Two rats from the UC-fragment/AFC-control group and one rat from the AP-fragment/AFC group became unstable in the restraining cage during Stage 2 of testing. Thus these three rats were ruled out of the study in Stage 2. Reliable results were obtained from 15 rats of the UC-fragment group and 17 rats of the AP-fragment group.

3.2.1. UC-fragment group
The effects of CS–US pairing on UC-fragment-induced PPI are shown in Fig. 5. For the AFC subgroup
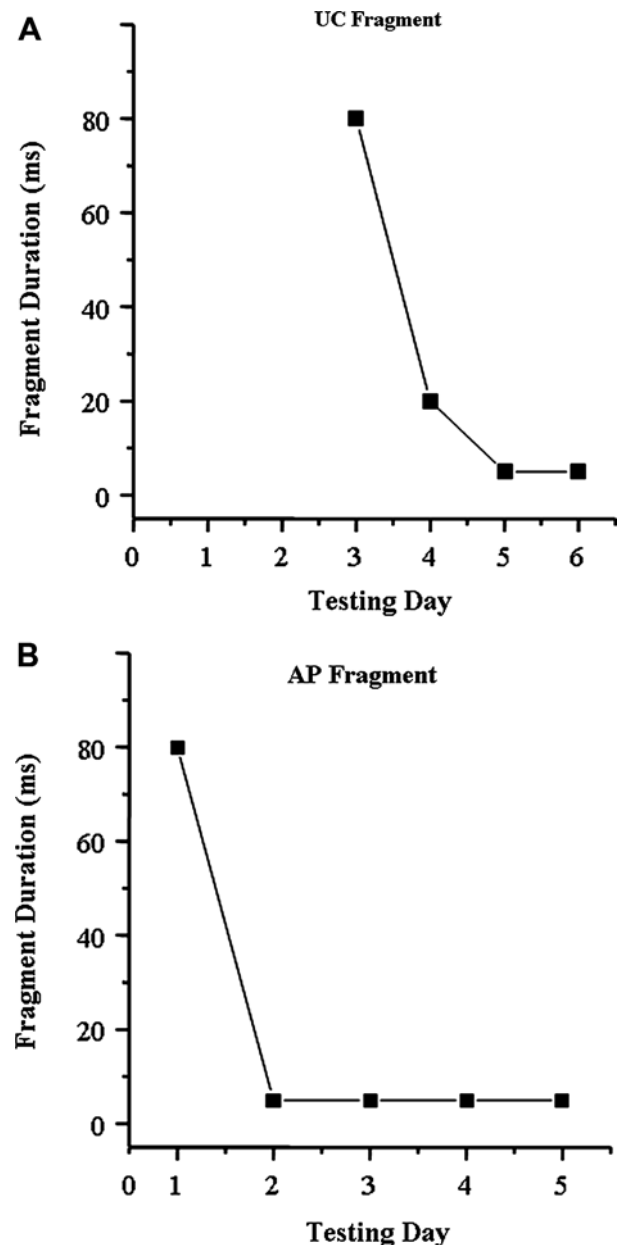

Fig. 4. Effects of the number of testing days during perceptual learning on the fragment detection threshold. Panel A: Rats were unable to detect any UC fragments (up to 80 ms) on Days 1 and 2, but were able to detect the 80-ms fragment on Day 3 and the 20-ms fragment on Day 4. On Days 5 and 6, rats were able to detect the 5-ms UC fragment. Panel B: Rats were able to detect the 80-ms fragment on Day 1 and the 5-ms fragment thereafter.

(Fig. 5a), following precisely temporal pairing of the 100-ms UC fragment with footshock, PPI was markedly enhanced. A 5 (fragment duration) by 2 (before and after pairing) two-way repeated ANOVA shows that the interaction between fragment duration and CS–US pairing was not significant ($F_{4,64} = 1.203$, MSE = 136.133, $p = 0.286$), the main effect of fragment duration was significant ($F_{4,64} = 8.095$, MSE = 859.029, $p < 0.001$), and the main effect of CS–US pairing was significant ($F_{1,16} = 7.391$, MSE = 3789.140, $p = 0.015$). The results suggest that the
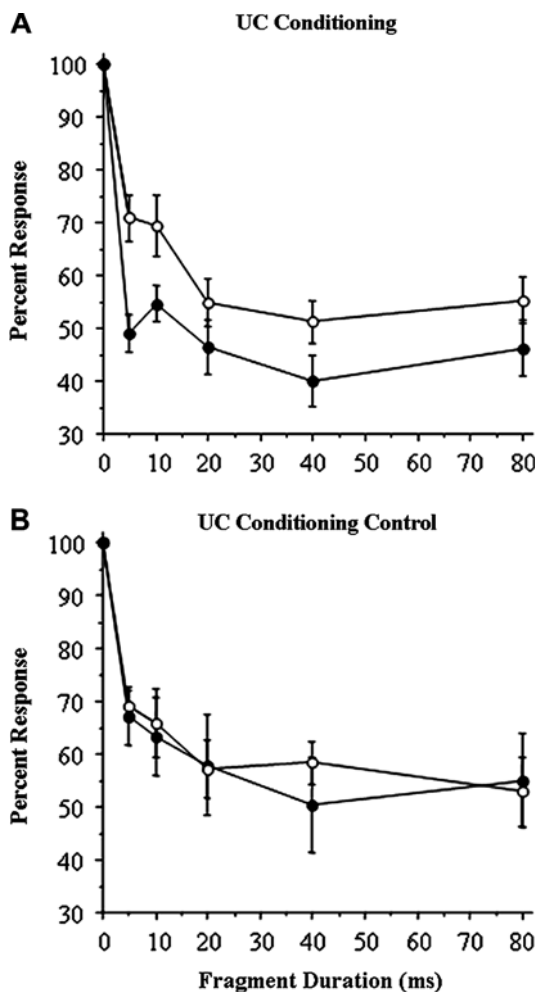
Fig. 5. The mean percent startle responses as the function of the duration of the UC fragment prepulse before and after the pairing of the 100-ms UC fragment with footshock in both the group of auditory fear conditioning (AFC) (Panel A) and the group of AFC-control (Panel B). Open circles indicate normalized startle responses before the pairing of the 100-ms UC fragment with footshock; close circles indicate normalized startle responses after the pairing of the 100-ms noise fragment with footshock. Error bars indicate the standard error of the mean.

precise CS–US pairing significantly enhanced PPI. The baseline startle responses (when the UC fragment duration = 0 ms) were not affected by the CS–US pairing ($F_{1,16} = 0.488$, MSE = 1,990,152.219, $p = 0.495$), indicating that the precise CS–US pairing did not affect baseline startle responses.

For the effect of temporally random pairing of the 100-ms UC fragment with footshock in the UC-control group (Fig. 5b), a 5×2 two-way repeated ANOVA shows that the interaction between fragment duration and CS–US pairing was not significant ($F_{4,40} = 0.448$, MSE = 45.917, $p = 0.773$), the main effect of fragment duration was significant ($F_{4,40} = 4.791$, MSE = 490.975, $p = 0.003$), and the main effect of CS–US pairing was not significant ($F_{1,10} = 0.051$, MSE = 52.318, $p = 0.825$). The baseline startle response was not significantly affected by the CS–US pairing ($F_{1,10} = 0.140$, MSE = 174,243.000, $p =$
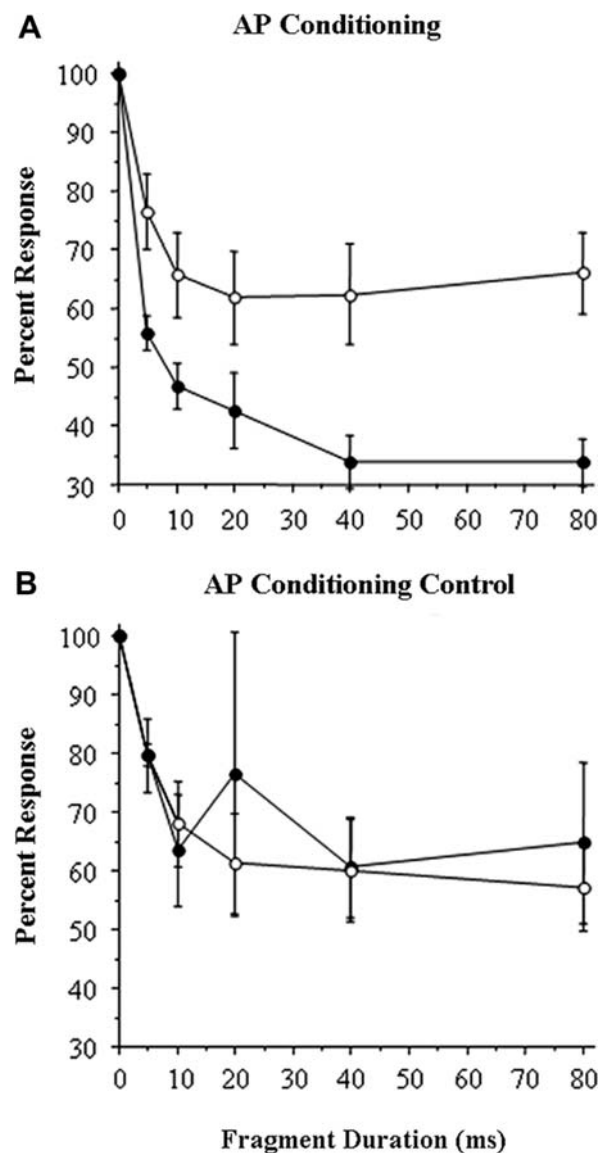

Fig. 6. The mean percent startle responses as the function of the duration of the AP fragment prepulse before and after the pairing of the 100-ms AP fragment with footshock in both the group of AFC (Panel A) and the group of AFC-control (Panel B). Open circles indicate normalized startle responses before the pairing of the 100-ms AP fragment with footshock; close circles indicate normalized startle responses after the pairing of the 100-ms noise fragment with footshock. Error bars indicate the standard error of the mean.

0.716). Thus temporally random paring of the UC fragment with footshock affected neither UC-fragment-induced PPI nor baseline startle.

3.2.2. AP-fragment group

The effects of CS–US pairing on AP-fragment-induced PPI are shown in Fig. 6. For the AFC subgroup (Fig. 6a), following precisely temporal pairing of the 100-ms AP fragment with footshock, PPI was markedly enhanced. A 5 (fragment duration) by 2 (before and after pairing) two-way repeated ANOVA shows that the interaction between fragment duration and CS–US pairing

treatment was significant ($F_{4,56} = 2.754$, MSE = 147.016, $p = 0.037$), the main effect of fragment duration was significant ($F_{4,56} = 15.595$, MSE = 832.528, $p < 0.001$), and the main effect of CS–US pairing was significant ($F_{1,14} = 8.674$, MSE = 11,503.115, $p = 0.011$). These results suggest that the precise CS–US pairing significantly enhanced PPI at some fragment durations. The baseline startle responses were not affected by the CS–US pairing ($F_{1,14} = 0.915$, MSE = 2,313,509.446, $p = 0.355$), suggesting that the precise CS–US pairing did not affect baseline startle responses.

For the effect of temporally random pairing of the 100-ms AP fragment with footshock in the AFC-control subgroup (Fig. 6b), a 5×2 two-way repeated ANOVA shows that the interaction between fragment duration and CS–US pairing was not significant ($F_{4,64} = 0.608$, MSE = 271.056, $p = 0.658$), the main effect of fragment duration was significant ($F_{4,64} = 2.498$, MSE = 1113.086, $p = 0.051$), and the main effect of CS–US pairing treatment was not significant ($F_{1,16} = 0.086$, MSE = 324.705, $p = 0.773$). Also, the baseline startle response was not significantly affected by the CS–US pairing ($F_{1,16} = 1.841$, MSE = 6,809,587.587, $p = 0.194$). Thus temporally random pairing of the AP fragment with footshock affected neither AP-fragment-induced PPI nor baseline startle.

4. Discussion

The results of the present study show that when identical (correlated) broadband noises with the zero inter-sound delay were presented by two spatially separated loudspeakers, a sudden and brief change of the correlation between the two noise sources (a UC fragment or an AP fragment) suppressed the ASR. The results suggest that rats can detect a UC fragment and an AP fragment.

4.1. The effect of the duration of the correlation change

As mentioned in the introduction, PPI has been used as a measure of the behavioral salience of prepulse stimulus. In the present study, suppression of the ASR by the inserted UC or AP fragment largely depended on the duration of the fragment, suggesting that the salience of the sound-correlation changes is determined by the duration of the change. As the noise fragment duration increased from 5 ms to 40 ms, the suppressive effect on the ASR progressively increased.

Previous studies using the PPI paradigm have shown that the duration threshold of detecting an energy gap for both rats and mice is about 2–5 ms (Barsz et al., 1998; Hoffman and Ison, 1980; Leitner and Girten, 1997; Leitner et al., 1997; Zou et al., 2006). In the present study, the suppressive effect was significant when the fragment duration was 5 ms or longer after perceptual learning. Thus rats are able to detect a considerably short dynamic change in the correlation between two sound sources.

4.2. The effect of perceptual learning

During the perceptual learning stage of this study, as the number of testing days increased, the suppressive effect of either a UC or an AP fragment on the ASR increased. Rats were unable to detect any UC fragments (up to 80 ms) on Days 1 and 2, but were able to detect the 80-ms fragment on Day 3 and even shorter fragments on Day 4. On Day 5, rats were able to detect the 5-ms UC fragment. For AP fragment detection, rats were able to detect the 80-ms fragment only on Day 1 but the 5-ms fragment thereafter. These results indicate that rats' ability of detecting uncorrelated noise fragments and that of detecting anti-phase noise fragments improve as they go through more testing sessions, showing a marked perceptual learning effect.

Experience-induced plasticity of PPI has interested people for over two decades (e.g., Crofton et al., 1990; Gewirtz and Davis, 1995; Plappert et al., 2004, 2006; Reijmers and Peeters, 1994; Wu et al., 1984; Zou et al., 2006). The PPI model established in the present study is useful for studying PPI plasticity induced by cognitive manipulations. Particularly, when the UC fragment is used as the prepulse, the monotonic enhancement of PPI over 5-6 days provides a unique chance for investigating the more detailed temporal features of the mechanisms underlying perceptual learning.

4.3. The effect of AFC

In the present study, PPI was significantly enhanced following temporally pairing the 100-ms UC or AP fragment with footshock in a precise manner. The results indicate that when a prepulse stimulus becomes conditioned and biologically meaningful following AFC, it elicits a greater gating effect compared to when it has not been conditioned. Previous human studies have shown that the depth of the processing of prepulse stimulus is highly correlated with the strength of PPI (Filion and Ciranni, 1994; Mussat-Whitlow and Blumenthal, 1997; Norris and Blumenthal, 1996; Perlstein et al., 1993). Thus the results of the present study suggest that sensory processing of the correlation change between two spatially separated sounds is enhanced by AFC.

For both the stage of perceptual learning and the stage of emotional learning, the 6 fragment-duration conditions (fragment duration = 0 ms, 5 ms, 10 ms, 20 ms, 40 ms, or 80 ms) were presented in a random order in each testing session. The amplitude of startle under the 0-ms condition was measured at each testing session through the whole learning course, and treated as the baseline response for normalizing responses under other conditions. We examined the baseline startle responses for each group and found that neither perceptual learning nor AFC had a significant effect on the baseline responses. Thus the enhancement of the PPI caused by perceptual learning or AFC was not due to changes of the ASR in each of the two stages of learning.

It has been well documented that attention enhances PPI in humans (Bohmelt et al., 1999; Filion and Ciranni,

1994; Filion and Poje, 2003; Filion et al., 1993; Heekeren et al., 2004; Jennings et al., 1996; Schell et al., 1995; Schell et al., 2000; Thornea et al., 2005). One of the possible explanations of the PPI enhancement observed in the present study is that conditioning the correlation change between sounds by footshock facilitates rats' attention to the UC or AP noise fragment, and facilitated attention to the prepulse signal enhances PPI. This explanation is consistent with the results of a human imaging study showing that in a PPI testing paradigm, greater blood oxygen level-dependent (BOLD) responses occurred in the attention-related anterior and mediodorsal thalamic nuclei when human subjects listened to attended prepulse tones than when they listened to ignored prepulse tones, and startling stimulus alone did not elicit such responses (Hazlett et al., 2001).

4.4. Differences between detection of the UC fragment and detection of the anti-phase fragment

Unlike the detection of an energetic gap, which mainly depends on the monaural process, the detection of a change in correlation between two spatially separated sources in the azimuthal plane may depend on both binaural processing and monaural processing. In the present study, there were differences in the rat's PPI performance between the two types of prepulse stimuli (UC fragment, AP fragment). In the early phase of perceptual learning, rats detected the AP fragment more easily than the UC fragment. Rats were also quicker in learning to detect small-duration AP fragments than learning to detect small-duration UC fragments. The gradually enhanced UC-induced PPI over a few days during perceptual learning can be particularly used as a model for studying detailed processes in cognitive modulations of PPI. In the stage of AFC, temporally pairing the noise fragment with footshock in the precise manner caused a greater enhancement of PPI when the prepulse was the AP fragment than when the prepulse was the UC fragment. Thus for the purpose of studying how emotional learning affects PPI, using an AP fragment as the prepulse would be more appropriate than the using a UC fragment.

Theoretically, when correlated noise sounds coming from the two spatially separated sources arrive to a point of the midline between the sources, the in-phase sound waves at the point sum up and the sound level measured in the midline is higher than that when independent noise sounds are delivered from the two sources. On the contrary, when AP fragments coming from the two spatially separated sources arrive to a point of the midline, sound waves from the two sides cancel each other, making the AP fragment ''gap like'' at middle points. In the present study, however, due to the rat's interaural distance (over 3 cm, Kelly and Phillips, 1991), the positions of the rat's ears were not on the midline, waveform summation and cancellation would not be fully achieved. Thus rats would detect the AP fragment and UC fragment using both inter-aural correlation cues and spectral cues. However, the weighting of using each of these cues might be different between AP-fragment detection and UC-fragment detection.

In summary, the present study established a new behavioral model by using the PPI paradigm for studying the detection of correlation changes between two spatially separated sound sources. Using this model, the present study shows that rats have an ability of detecting correlation changes between broadband noise sounds coming from two spatial locations in the azimuthal plane, and the ability can be improved by both perceptual learning and emotional learning. This behavioral model has potential applications for studying the interactions between auditory perception, sensorimotor gating, and perceptual or emotional learning in both laboratory animals and human subjects.

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J. Huang et al. / Hearing Research 223 (2007) 1–10

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