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ORIGINAL ARTICLE

Fear leads to a deficit of prepulse inhibition of blink reflex in healthy humans

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Abstract

Objective We aimed to analyze whether or not fear conditioning exerts an effect on prepulse inhibition (PPI) of blink reflex (BR). To create fear conditioning, we used fearful faces. Since fearful faces lead to a specific set of fear conditioning, we hypothesized PPI of BR would change under the observation of fearful faces.

Method We included 17 healthy subjects with a mean age of 30.8 ± 6.9 years and seven healthy subjects with a mean age of 57.7 ± 7.3 years between January 2018 and June 2018 and recorded PPI of BR. The recordings were done before observation of any image, during observation of images, and immediately after observation of images. Observation of images included observation of fearful faces for 30 s and a neutral image of a white screen for 30 s (in a randomized order).

Results There was a R2-PPI deficit during observation of fearful faces in each group whereas R2-PPI fully developed at other time points. R1 amplitude and R2 magnitude were lower during observation of any image compared with baseline and post-observation time points.

Conclusion In conclusion, a deficit of R2-PPI develops during observation of fearful faces in humans which is probably related to activation of the amygdala.

Keywords Blink reflex · Blink reflex prepulse inhibition · Fearful faces

Introduction

Prepulse modulation is an electrophysiological method in which a change in the magnitude of the response occurs when it is preceded by a weaker stimulus. To obtain prepulse modulation (PPM), auditory startle response (ASR), blink reflex (BR), masseter inhibitory reflex, or startle response to somatosensory inputs has been used [1–3]. The usual change in the late, bilateral BR response (R2) after prepulse stimulus is the reduction of magnitude which is also called prepulse inhibition (PPI). PPI is generated at the level of the pedunculopontine nucleus. It reflects the filtering activity of a network between the basal ganglia and the brainstem reticular formation [4, 5]. It provides processing of the first input without interference of the consecutive one. However, it is not a pure automatic process. Top-down modulation of PPI is operative in humans, especially when prepulse stimulus is applied at longer intervals prior to the test stimulus (ISIs > 120 ms) [6, 7]. In humans and animals, effect of prepulse stimulus is modulated by cognitive processes such as attention [8, 9].

Fear conditioning is a model derived from the initial experiments of Pavlov in 1927. In the experiments using fear conditioning, different variants of conditioning stimuli have been employed [10]. The response to fear conditioning may involve motor, neuroendocrine, or autonomic changes [11]. From the electrophysiological perspective, the effect of fear conditioning on ASR is well known in humans or animals [12]. Fear leads to the potentiation of ASR in healthy humans. In humans, immediate reaction to fear is motor freezing [13] whereas potentiation of motor-evoked potentials occurs within hundreds of seconds [14]. Fear may also change information processing. The motor and autonomic responses to fear are mediated by the amygdala [11].We recently showed the role of the amygdala in sensory processing [15]. Thus, we aimed to analyze whether or not fear conditioning exerts an effect on PPI of BR. To create fear conditioning, we used fearful faces. We hypothesized PPI of BR would change under the observation of fearful faces. We analyzed the same paradigm in younger and older populations to observe the effects of aging.

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Subjects and methods

Subjects We included consecutive 17 healthy subjects with a mean age of 30.8 ± 6.9 years and seven healthy subjects with a mean age of 57.7 ± 7.3 years between January 2018 and June 2018. The participants who volunteered for the study were working in our electrophysiology laboratory or in the neurology clinics; however, they were naïve to fearful faces. There were 11 (64.7%) and 3 (42.8%) women, respectively. None of the participants had anxiety, psychosis, or depression. Participants with any disorders causing biases in electrophysiological investigations were excluded from the study. The study was approved by the local ethical committee.

Method Recordings were done using surface electrodes and Neuropack Sigma MEB-5504k (Nihon Kohden Medical, Tokyo, Japan) while patients were relaxed and sitting in an armchair. Blink reflex and BR-PPM were recorded in all participants. The Ag-AgCl pair of cutaneous recording electrodes was placed on bilateral orbicularis oculi muscles according to the previous standards. Unilateral recordings were done after stimulation of the ipsilateral trigeminal nerve. Four trials were performed under each condition. Responses were rectified and averaged. The ground electrode was placed on the forehead. The analysis time, sensitivity, and filter settings were 30 ms/division, 100 μV/division, and 3-kHz and 20-Hz high and low cut-off frequencies, respectively.

Blink reflex A 0.2-ms electrical stimulation of the supraorbital branch of the trigeminal nerve was used at the supraorbital notch (test stimulus alone). The intensity of the stimulus was five times the intensity of the R2 threshold.

Prepulse modulation of blink reflex The median nerve was stimulated (100 ms) (conditioning, prepulse stimulus) before the stimulus to the supraorbital nerve (test stimulus). For this purpose, an electrical stimulus of 0.2 ms in duration and at the intensity of the perception threshold of the second finger was used.

Experiment A trial included observation of fearful faces for 30 s and a neutral image of a white screen for 30 s. For fearful faces, recordings which included actors displaying fearful expressions (Karolinska Directed Emotional Faces database) [16] were shown using 13.1-in. ASUS X550C series model laptop under maximal luminance measurements. Four recordings during each image were collected. Each trial was shown consecutively but with changing orders to provide randomization and to prevent interference with habituation. The distance between the eyes of participants and the screen was 1 m. The physician responsible for the recordings and the measurements was blind to the images throughout the examination.

The recordings were done:

i. Before observation of any image
ii. During observation of images (with changing order)
iii. Immediately after observation of images

Statistical analysis We measured the following parameters:

i. Onset latencies (ms) of the R1 and R2 responses,
ii. Peak-to-peak amplitudes (μV) of the R1 responses,
iii. Area under the curve (AUC) of R2 responses

For prepulse modulation, the following calculation was done:

$$\text{Prepulse modulation (at 100 ms)} = \text{R2 AUC after conditioning plus test stimulus} / \text{R2 AUC after test stimulus alone} \times 100.$$

A similar formula was used to calculate percentage change of R1-prepulse modulation.

Each BR parameter and percentage change of prepulse modulation was compared between the following time points:

1. Before observation of images vs during observation of fearful faces
2. Before observation of images vs during observation of a white screen
3. During observation of fearful faces vs during observation of a white screen
4. Before observation of images vs after observation of images.

To understand the changes after prepulse stimulus, R1 amplitude and R2 AUC after test stimulus alone and after conditioning plus test stimulus were also compared within each time point.

Data analyses were performed using the SPSS 20.0 software statistical package. We first analyzed normality using a Shapiro-Wilk test. Then, since the distribution was nonnormal, we used a Wilcoxon signed-rank test or Friedman's test depending on the number of groups. p value < 0.05 was considered significant.

Results

Blink reflex R1 and R2 responses during baseline condition were obtained in all subjects. Baseline values were normal according to our laboratory standards. Mean R1 amplitude at baseline was significantly lower in the older population compared with the younger population ($p = 0.001$). Other parameters of BR were similar between groups. In the younger age group, when we compared R1 amplitudes recorded during all time points, there was a significant difference ($p = 0.018$). Post hoc analyses

revealed significantly lower amplitudes during observation of fearful faces, or a white screen, compared with the time point before watching images (Table 1). Immediately after the removal of the image, R1 amplitude was similar to that recorded during baseline condition. Similarly, R2 AUC was also lower during observation of images of fearful faces, or a white screen, compared with the baseline time point (Table 1). It also returned to the baseline values immediately after the removal of the images. The R1 and R2 latencies obtained during observation of any image were similar to those obtained during the baseline time point.

In the older age group, there was a trend for lower R2 AUC during observation of images of fearful faces, or a white screen compared with the baseline time point (Table 2).

Prepulse modulation of blink reflex In the younger population, during observation of fearful faces, there was a small but significant reduction of R1 amplitude after prepulse stimulation compared with the recordings after test stimulus alone whereas there was no significant change of R1 amplitude after prepulse stimulation compared with that obtained after test stimulus alone at other time points (Fig. 1a). Percentage change of R1-prepulse modulation was similar between baseline and any of the other time points. In the older population, there was no significant change of R1 amplitude after prepulse stimulus compared with test-only stimulus at any time point.

Considering R2 magnitude, there was a reduction of R2 AUC after prepulse stimulation at all time points except the recordings during observation of fearful faces, in which condition there was a PPI deficit in the younger population (Fig. 1b). PPI% during observation of fearful faces was significantly higher (PPI deficit) compared with the baseline condition ($p = 0.028$) whereas PPI% at other time points was similar to baseline. In the older age group, R2 magnitude after prepulse stimulus was also significantly reduced compared with test stimulus alone at baseline ($p = 0.018$). There was PPI deficit only during observation of fearful faces. At other time points, R2-PPI was evident (Fig. 1b).

There were no significant changes in the latencies. Figure 2 shows graphical representation of R1 and R2 latencies at all time points.

Discussion

The major finding in our study is the R2-PPI deficit during observation of fearful faces in younger or older population. Other findings are the reduction of R1 amplitude and R2 magnitude during observation of any image compared with baseline and post-observation time points.

In humans or animals, PPI is generally obtained by applying a brief prepulse stimulus before the salient auditory stimulus. The generator of PPI is governed by the inhibitory cholinergic projections from the pedunculopontine tegmental nucleus in the pons [4, 5, 17]. A functional neuroimaging study showed changes of the orbitofrontal cortex, cerebellum, thalamus, and anterior cingulate cortex activity during prepulse modulation of startle reflex in humans [18].

Modification of PPI under different tasks is an approach to understand the suprasegmental influence. In rats, fear-conditioned prepulse and a noise masker facilitated selective attention to the prepulse and enhanced PPI whereas motivated attention reduced it [19]. In humans, stress induced by performing a difficult task or an experiment involving a threat clue reduced PPI [20, 21] or directed attention enhanced PPI when there was enough time for the attentional mechanisms to exert an influence [22]. Herein, we showed the development of PPI deficit specifically after fearful faces. Other images did not lead to any change in the PPI although they changed the BR magnitude. We previously demonstrated a similar PPI deficit in the peripersonal space [23]. We attributed PPI deficit in the peripersonal space to the tonic top-down modulation exerted by the association areas such as the premotor cortex and ventral intraparietal area which are thought to encode the peripersonal space in the brain. PPI deficit under fear conditioning also suggests a possible modulatory influence by the anatomical structures in fear conditioning upon PPI. Interestingly, the findings of this study were just the opposite of those found in animals since the fear conditioning leads to an enhanced PPI in animals [24]. In animals, inactivation of the basolateral amygdala by local injections leads to the impairment in PPI [25].

Table 1 Findings of blink reflex after test stimulus and after prepulse-test stimuli under each condition in the younger population

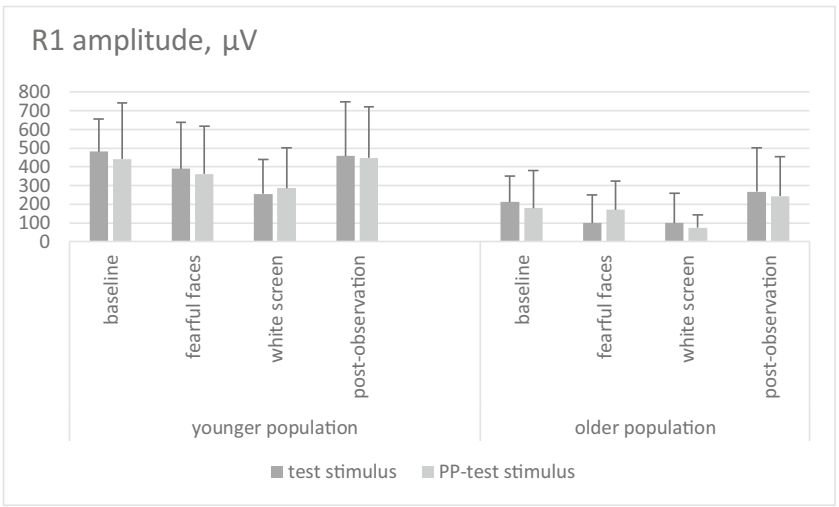
Time points	Baseline	Fearful faces	White screen	Post-observation	*p*
BR parameters					
R1 latency, ms	10.3 ± 1.1	10.0 ± 0.6	10.2 ± 0.8	10 ± 0.9	0.767
R1 amplitude, μV	481.1 ± 174	390.2 ± 246.8	254.6 ± 183.4	457.2 ± 290.3	0.018*
R2 latency, ms	32.0 ± 3.2	34.4 ± 3.7	32.9 ± 3.2	32.9 ± 3.2	0.327
R2 AUC, mV	4.4 ± 2.4	2.7 ± 2	3.2 ± 2.2	4.6 ± 2.6	0.003*
After prepulse stimulation					
R1 latency, ms	10.1 ± 0.8	10.2 ± 0.7	10.1 ± 0.8	10.3 ± 0.6	0.469
R1 amplitude, μV	441.2 ± 300.2	361.6 ± 255.1	285.9 ± 215.7	448.2 ± 271.4	0.692
R2 latency, ms	35.2 ± 4.2	35.1 ± 3.9	36.1 ± 4.2	32.7 ± 1.7	0.862
R2 AUC, mV	1.3 ± 1.3	2.3 ± 3.9	1.8 ± 2.8	1.9 ± 1.9	0.663

*$p < 0.005$

Table 2 Findings of blink reflex after test stimulus and after prepulse-test stimuli under each condition in the older population

Time points	Baseline	Fearful faces	White screen	Post-observation	*p*
BR parameters					
R1 latency, ms	11.1 ± 0.9	10.8 ± 0.5	10.8 ± 0.8	10.8 ± 0.9	0.965
R1 amplitude, μV	212.1 ± 137.2	98.8 ± 156.7	100.0 ± 160.0	266.2 ± 233.9	0.471
R2 latency, ms	33,6 ± 4.5	37.3 ± 4.2	37.3 ± 4.9	35.4 ± 3.3	0.468
R2 AUC, mV	2.8 ± 1.1	1.4 ± 1.1	1.2 ± 0.3	2.9 ± 1.3	0.051
After prepulse stimulation					
R1 latency, ms	10.6 ± 0.8	10.0 ± 0.5	10.3 ± 0.6	10.9 ± 0.7	0.392
R1 amplitude, μV	178.7 ± 200.1	170.0 ± 155.2	73.5 ± 70.2	242.5 ± 210.6	0.122
R2 latency, ms	37.7 ± 5.3	34.6 ± 3.8	37.2 ± 5.2	37.2 ± 2.8	0.934
R2 AUC, mV	0.5 ± 1.1	1.0 ± 1.6	0.7 ± 1.0	1.1 ± 1.7	0.431

Fear may be created by fear-related music or fearful images. The relationship of fear and startle response has been relatively well defined, and most of the studies covering fear behavior and electrophysiology have used ASR in their paradigm. After fear conditioning, magnitudes of ASR get bigger or latencies shorten, indicating hyperexcitable ASRs [26]. The sizes of motor-evoked potentials may also enhance after fear-related music or fearful images [14]. All these changes are accepted as electrophysiological counterparts of fight and flight reaction. After observation of fearful faces in humans, there occurs an increased activity of the



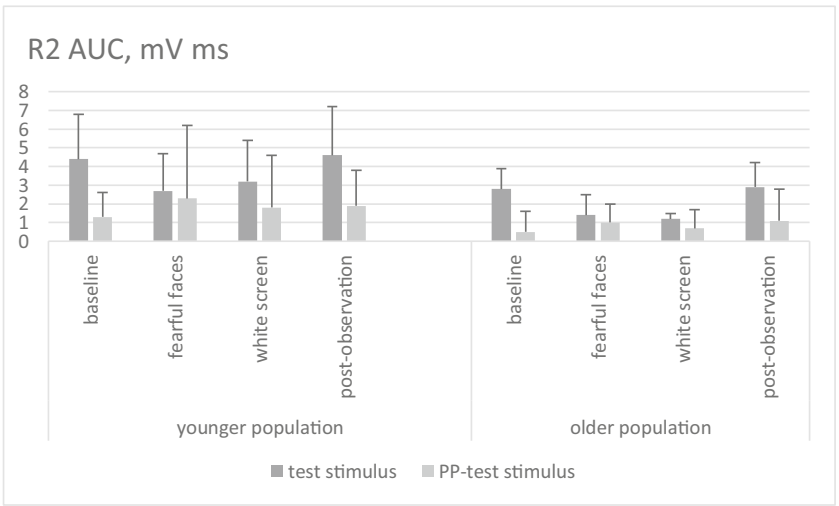
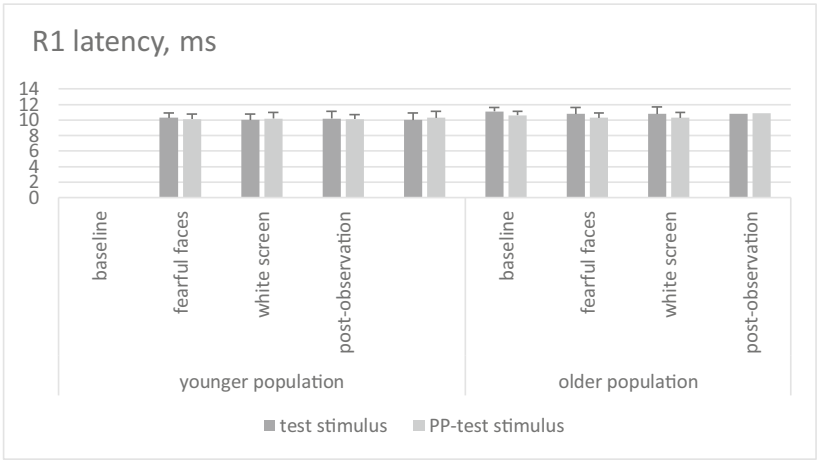


Fig. 1 **a** Mean R1 amplitudes after test stimulus alone and after prepulse plus test stimulus at all time points. **b** Mean R2 AUC after test stimulus alone and after prepulse plus test stimulus in all time points (error bars represent standard deviations; R2 AUC, R2 area under the curve; PP, prepulse stimulation)



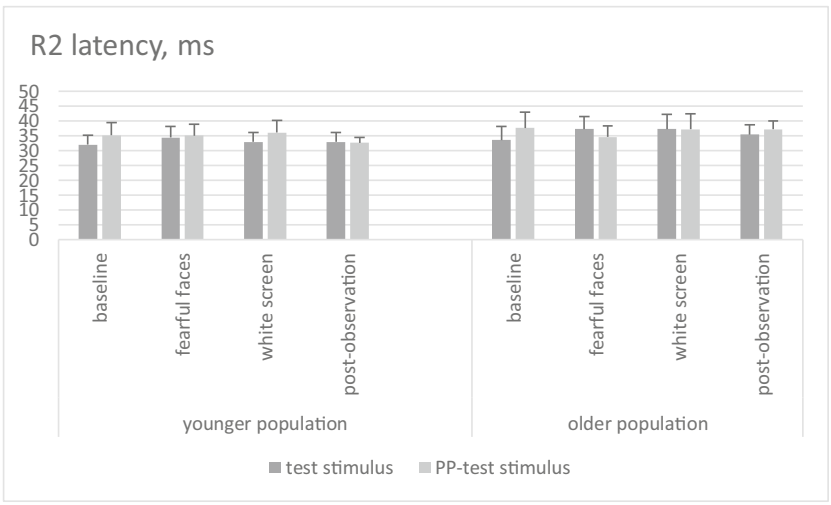


Fig. 2 **a** Mean R1 latency after test stimulus alone and after prepulse plus test stimulus at all time points. **b** Mean R2 latency after test stimulus alone and after prepulse plus test stimulus at all time points (error bars represent standard deviations; PP, prepulse stimulation)

right amygdalae and bilateral hippocampi [27]. Thus, our study suggests PPI deficit develops after the activation of the amygdala by the fearful faces in healthy humans. Activation of the amygdala in reaction to fearful faces occurs in all age groups [28]. We also analyzed groups with different ages and the PPI deficit developed by fear conditioning in any age group.

There may be two reasons of the difference between animals and humans: (i) there is a differential modulation of PPI by different amygdala nuclei in humans similar to that shown by Du and colleagues in animals [24], or (ii) a deficit in PPI develops after activation of limbic structures similar to that seen after limbic seizures in amygdala-kindled rats [29]. Thus, overstimulation or activation of the amygdala may also lead to the PPI deficit.

According to the protective hypothesis, PPI provides protection of ongoing sensory processing. Analogously to the PPI deficit in the peripersonal space, we may speculate that ongoing peripheral sensory processing is interrupted and response on the eye muscle is more evident in the presence of fearful faces.

Although observation of fearful faces causes a reduction in the facilitation of R1 after prepulse stimulus, the change was not as evident as the change in R2-PPI. R1 is mediated by an oligosynaptic circuit between the trigeminal sensory nuclei and facial motoneurons in the pons, and the circuit of R1 does not involve the ponto-medullary reticular formation [30]. Thus, R1 facilitation is not subject to the suprasegmental modulatory influences as much as R2-PPI in healthy humans.

Regarding BR magnitude, we found a nonspecific reduction of R1 amplitude and R2 magnitude during observation of any image. The nonspecific reduction in the size of BR may be related to attention to the screen. However, increased BR magnitude was consistent across a range of stimulus modality and task difficulty conditions requiring attention in previous studies [31]. Light itself is able to change the size of BR [32]. We think that the reduction in BR size is possibly related to a strategy, suppression of blinks, in the presence of visual stimuli, because it was reversed immediately after the removal of the screen.

There were certain limitations to this study. To record PPI while observing happy faces would provide more information about the underlying physiology.

Conclusion

A deficit of R2-PPI develops during observation of fearful faces in humans which is probably related to amygdala activation. The amygdala is affected in conditions such as Alzheimer's disease and temporal lobe epilepsy. To study the method in these disorders or in other conditions with PPI deficit in the future may be interesting.

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Compliance with ethical standards All participants provided informed consent.

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