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ORIGINAL PAPER

Ulrich Meincke · Dina Mörth · Tatjana Voß · Euphrosyne Gouzoulis-Mayfrank

Electromyographical differentiation between the acoustic blink and startle reflex

Implications for studies investigating startle behavior

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Abstract Recent evidence suggests that electromyographic activity in the orbicularis oculi muscle occurring in response to sudden acoustic stimuli consists of two overlapping components: the blink and the startle reflex.

The aim of the present study was to identify these two components in acoustically elicited eyeblink responses and to analyze their differential modulation by weak acoustic prepulses. The prevalence, latency and amplitude characteristics of double EMG peaks in pulse-alone and prepulse-pulse trials (PP) with 30 ms and 100 ms interstimulus intervals were assessed in 16 healthy volunteers.

EMG responses with two peaks were registered in 42.6 % of the pulse-alone trials and in 56.2 % of the PP30 and 48.7 % of the PP100 trials, respectively. Prepulse inhibition of the amplitude was greater for the second peak (14.2 % (P2) vs. –11.5 % (P1) in PP30 trials; 62.6 % (P2) vs. 32.3 % (P1) in PP100 trials), resulting also in higher P1/P2 amplitude ratios in prepulse-pulse trials (P1/P2: 62.9 % in pulse-alone, 92.6 % in PP30 and 100.1 % in PP100 trials).

In conclusion, double peaks are a common phenomenon in human studies of acoustically elicited blink responses. It is postulated that the first peak represents the auditory blink reflex, whereas the second peak corresponds to the startle reflex, which may be more susceptible to prepulse inhibition. This complexity should be taken into account in clinical studies of the modulation of the startle reflex.

Key words blink reflex · prepulse-inhibition · schizophrenia · startle reflex

U. Meincke, MD (✉) · D. Mörth · T. Voß · E. Gouzoulis-Mayfrank
Department of Psychiatry and Psychotherapy
Technical University of Aachen
Pauwelsstraße 30
52057 Aachen, Germany
Tel.: +49-241/8089632
Fax: +49-241/8082401
E-Mail: umeincke@post.klinikum.rwth-aachen.de

Introduction

In humans, the startle reaction to sudden auditory stimuli of high intensity consists of a generalized motor response, predominantly in the upper half of the body [11]. The eyeblink component of the startle response can easily be measured as the EMG activity of the orbicularis oculi muscles using surface electrodes which can be used in investigations of startle excitability under different physiological and pathological conditions. In particular, the assessment of prepulse inhibition of the acoustic startle response represents an operational measure of sensorimotor gating and has become a valuable tool to investigate information processing deficits in various neuropsychiatric disorders [e. g., 1, 2, 9,17–19].

Nevertheless, recent neurophysiological studies suggest that the activity in the orbicularis oculi muscle consists of temporally overlapping components: the blink and the startle reflex [3, 21]. Already in 1982, Davis [6] reported that blink responses with different latencies and different neural substrates may be evoked after auditory stimulation. Nearly 10 years later Brown [3] showed that in humans the early portions may represent the auditory blink reflex, which is a protective reflex for the eye only and is not part of the generalized auditory startle reflex. For this assumption he presented the following arguments: 1) The auditory blink reflex can be registered without any other manifestation of the startle reaction: after repetitive stimulation and habituation of the startle reflexes in other craniocervical muscles there is persisting EMG activity in the orbicularis oculi muscle of shorter and more constant duration. 2) The latency of this auditory blink reflex is much shorter than the onset latency of EMG activity in other cranial muscles despite their smaller distance to their innervations from the caudal brainstem. 3) These different components could be separated electromyographically in 36 % of the trials after acoustical stimulation [3].

With this neurophysiological background, our frequent observation of blink responses with two peaks in

human studies investigating auditory startle behavior may be relevant. Using the same, widely applied computerized system to investigate prepulse inhibition (PPI) of the startle reflex it has also been the experience of other groups to regularly observe two peaks in the smoothed EMG response of some subjects (M. Geyer, K. Abel, personal communication). To our knowledge, only one study has mentioned explicitly which peak was measured [19]. However, this uncertainty raises important questions for studies of startle behavior in psychiatric research. These questions concern the functional correlates of the two different peaks and the decision of which peak to measure in order to reliably investigate startle responses. It is highly probable that the two peaks represent the maximal amplitudes of the overlapping auditory blink and startle reflexes. However, it is not clear whether it is possible to differentiate between acoustic blink reflexes and startle reflexes in studies designed to assess prepulse inhibition. Moreover, it is not clear whether there is a differential prepulse modulation of blink and startle reflexes or whether these distinct reflexes share common physiological traits. In the present study, we address these questions by determining the prevalence, latency and amplitude characteristics of blink responses with two peaks in a sample of healthy subjects using stimulation and recording parameters commonly applied in studies of PPI.

Methods

Seventeen healthy volunteers were studied; one subject was excluded because of responses with very small amplitudes (see below). Thus, data are reported for a total of 16 subjects (8 women, 8 men) with a mean age of 31.4 ± 3.5 years (range: 26–36 years). They were recruited from the community and hospital staff of the University Hospital of Aachen. Exclusion criteria comprised a neurological or psychiatric disorder (according to DSM IV, axis I and II) or substance abuse during the previous two years. All subjects were free of medication and had no family history of an Axis I disorder in a first degree relative.

Electrophysiological examination was performed in a quiet room. Subjects were asked to relax and to look at a blank wall approximately 2 m in front of them while sitting comfortably in an armchair. They were told that the experiment was concerned with the behavior of simple reflexes and that during the session they would sometimes hear a noise through headphones that could be ignored. All subjects gave their informed consent to participate in the study.

Electromyographic activity was recorded from the right orbicularis oculi muscle with small Ag/AgCl surface electrodes filled with electrolyte paste and fixed below the right eye. The ground electrode was placed 2 cm below the right mastoid. Electrode resistances were less than 5 k Ω.

Reflex measures were carried out using a commercially available device (SR-Lab, San Diego Instr., San Diego, CA, USA). Via this computerized system EMG activity was recorded in 250 1-ms readings from pulse onset, bandpass filtered (1–1000 Hz), amplified, digitized and rectified. For analysis, the digital signal was smoothed by averaging 10 successive points. The acoustic stimuli were presented binaurally through headphones (TDH–39-P, Maico, Minneapolis, MN, USA).

After an acclimation period of 5 minutes to a 65dB (A) broadband noise which served as a continuing background noise during the session, 4 pulse-alone (PA) trials were presented in a first block in order to identify "non-responsive" subjects (mean peak amplitude of the entire response less than 25 digital units (0.19 mV)) and exclude them from further analysis. The reflex eliciting stimuli were white noise bursts with an intensity of 115 dB (A) and a duration of 20 ms. The second block consisted of 30 trials presented in a pseudorandomized order. Apart from 10 PA trials, there were 20 prepulse-pulse (PP) trials with a weak acoustic prestimulation (8 dB above background noise, 20 ms duration) being followed by the 115 dB stimulus. The interstimulus interval (ISI) from onset of prepulse to onset of stimulus was 30 ms or 100 ms (PP30 and PP100, 10 trials each). The intertrial intervals varied between 8 and 22 s.

Peak amplitudes, onset, offset and peak latencies were measured (Fig. 1). Peak amplitudes were detected within a time window of 30 to 95 ms following stimulus onset. We rated every deflection as a peak amplitude if it reached at least 15 % of the entire deflection. Responses with two peaks were identified and their amplitude ratios P1/P2 (percentage scores) were calculated in each trial. Onset and offset latency were defined as the time from stimulus to the beginning of the EMG deflection from baseline and the return to baseline, respectively. Responses were excluded if the baseline shift was greater than 50 units.

For each subject the mean peak amplitudes, peak latencies, amplitude ratios as well as onset and offset latencies were defined for each of the three different types of trials (i. e., PA, PP30, PP100) in the second block of the startle session. Thereafter, group means and standard deviations (SD) were calculated from this data. Prepulse inhibition (PPI) was defined as the percentage reduction of the peak amplitude in prepulse-pulse over pulse-alone trials (100 X (pulse-alone – prepulse-pulse) / pulse-alone).

One-factor ANOVAs and subsequent Tukey's studentized Range Tests were performed in order to assess the influence of the type of trial (PA, PP30, PP100) on the prevalence of double peaked responses, the peak latencies, the peak amplitudes and the amplitude scores P1/P2 as well as the onset and offset latencies of the reflex responses. In addition, we used paired *t*-tests in order to compare the PPI of the first and second peak. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SAS (windows, version 6.0).

Results

Fig. 2 displays the data of prevalence and amplitude characteristics of EMG responses with double peaks. In each type of trials a marked prevalence of responses with two peaks was found, with a tendency to be higher in prepulse-pulse trials, especially in PP30 trials. Three of the 16 subjects displayed only responses with one peak. Three PP100 trials were excluded because they displayed responses with three peaks.

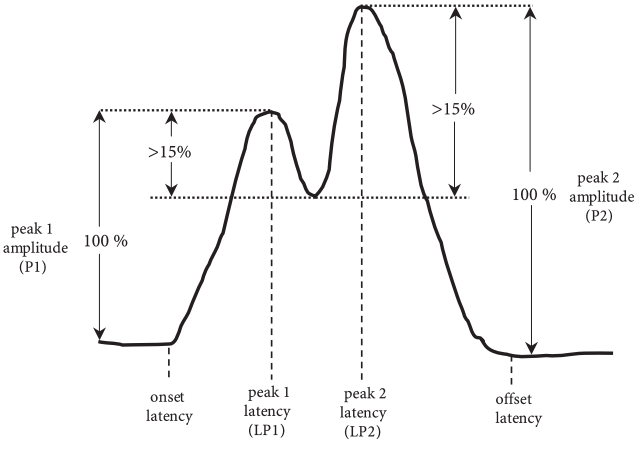

Fig. 1 Schematic illustration of applied amplitude and latency parameters in acoustic eyeblink responses.

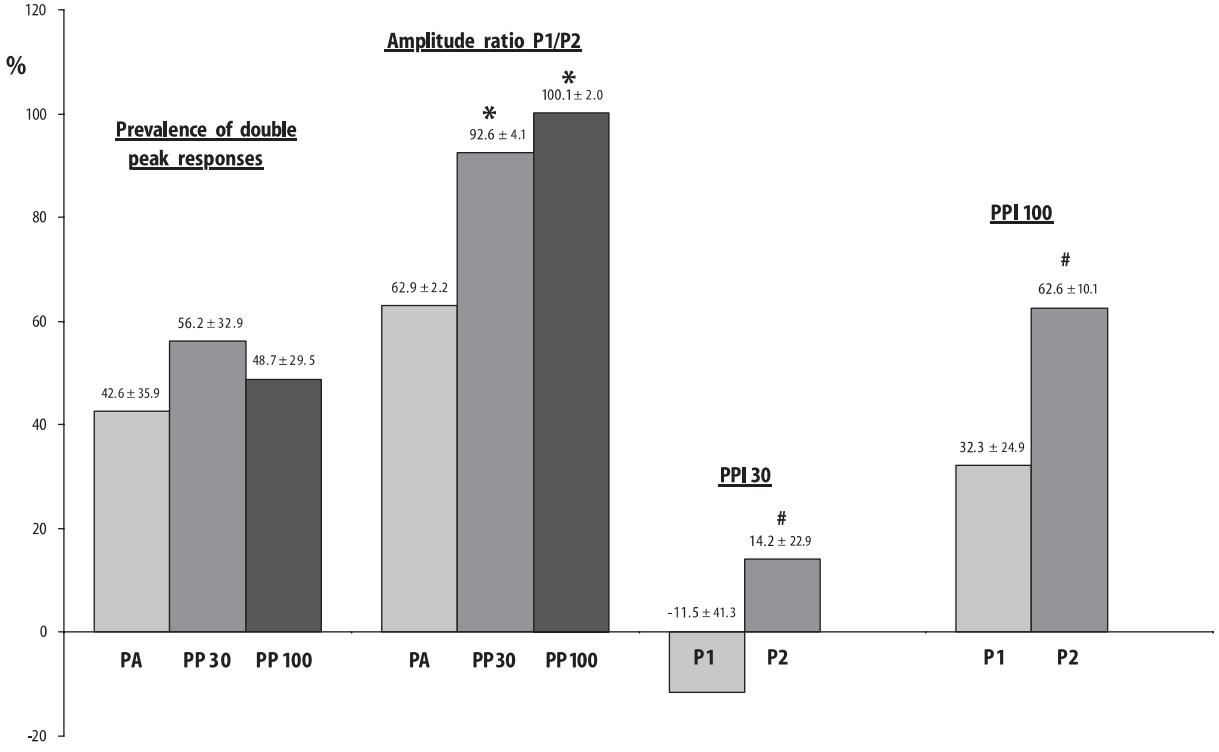

Fig. 2 Characteristics of double peaked (P1,P2) eyeblink responses in pulse-alone (PA) and prepulse-pulse trials with interstimulus intervals of 30 ms (PP30) and 100 ms (PP100). Means ± standard deviations of prevalence, amplitude scores P1/P2 and prepulse inhibition (PPI). * significant difference in comparison to pulse alone trials (ANOVA, Tukey's studentized range test), # significant difference in comparison to P1 (paired t-test) , ($p < 0.05$).

Prepulse inhibition of the second peak was greater than prepulse inhibition of the first peak (PP30 trials: $T = 2.69$, $p < 0.02$, PP100 trials: $T = 4.36$, $p < 0.001$, paired *t*-test). In pulse-alone trials the mean amplitude of the first peak was smaller than the mean amplitude of the second peak (amplitude ratio P1/P2 = 62.9 ± 22.1). In prepulse-pulse trials the amplitude ratio P1/P2 was significantly increased in comparison to pulse-alone trials ($F = 9.75$, df 2, 42, $p < 0.05$, ANOVA).

The mean latencies of both peaks appeared to be slightly shorter in prepulse-pulse trials compared to pulse-alone trials (Table 1); however, these differences did not reach statistical significance. The onset latencies of eyeblink responses were found to be significantly facilitated in PP30 and PP100 trials ($F = 5.58$, df 2, 45, $p < 0.05$, ANOVA). Offset latencies showed a nonsignificant shortening in PP30 trials and a significant reduction in PP100 trials of 10.3 ± 1.4 ms in comparison to pulse-alone trials ($F = 11.19$, df 2, 45, $p < 0.05$, ANOVA), (Table 1).

Table 1 Means and standard deviations of peak latencies in blink responses with two peaks and onset-, offset latencies of all trials.

	pulse alone	PP30	PP100
Latency peak 1 (ms)	46.1 ± 3.9	45.0 ± 3.5	44.5 ± 2.4
Latency peak 2 (ms)	66.6 ± 5.4	65.1 ± 4.3	64.6 ± 4.2
Onset latency (ms)	35.9 ± 3.9	33.0 ± 2.8*	32.1 ± 2.8*
Offset latency (ms)	87.6 ± 5.9	83.2 ± 5.2	77.4 ± 7.2*

* significant difference compared to pulse-alone trials ($p < 0.05$, ANOVA, Tukey's studentized range test)

Discussion

Modulation of blink responses has proved to be a valuable tool to operationally investigate attentional deficits and information processing dysfunctions in patients with different neuropsychiatric disorders [2, 15, 17–19]. In general, the acoustic response of the orbicularis oculi muscle is considered to be the most reliable component of the overall startle reaction. However, recent evidence suggests that the orbicularis oculi response includes an overlapping auditory blink reflex. This component is thought to be a protective brainstem reflex for the eye only. It is characterized by a shorter duration and onset latency compared to the true startle reflex and it can be registered in the orbicularis oculi muscle without concomitant EMG activity in other craniocervical muscles [3, 21].

Our data are in line with the results of Brown and coworkers [3]: Indeed, in our study with healthy volunteers we were able to differentiate two components of the acoustic blink response electromyographically in a considerable number of the trials. Our data support the hypothesis that the two components correspond to the blink and startle reflex, respectively. Theoretically, an

eyeblink response with two peaks should be expected when the two peak latencies differ considerably (Fig. 3a). Depending on the response configuration, the appearance of a response with three peaks, as observed in three PP100 trials, might also be possible (Fig. 3b). Otherwise, a response with one peak should emerge when a small reflex is obscured by a larger one (Fig. 3c) or when reflexes with similar latencies extensively overlap (Fig. 3d). Since latencies of both acoustic blink reflexes and startle reflexes show a marked variability, this waveform is supposed to be the most frequent one.

Based on previous results, we expected to elicit both the blink and startle reflex simultaneously in a remarkable number of trials by the use of 115 dB stimuli: Valls-Solé and collegues [21] consistently evoked just a blink reflex of a small amplitude by weak stimuli of 82 dB; in contrast, 130 dB pulses were needed to elicit a stable startle reflex of a sufficiently high amplitude that obscured the auditory blink reflex. In the present study we predicted that both the blink reflex and the startle reflex with moderate amplitudes would be elicited using 115 dB-stimuli – an intensity that is widely applied in studies investigating startle behavior.

Our observation of responses with two peaks is not an artifact. The applied automatic EMG system smoothes and rectifies the raw signal, and although components had to reach a marked amplitude (> 15 % of the entire deflection) in order to be rated in our study, double peaks were noticed in a remarkable number of trials.

It is a common phenomenon to elicit EMG activity of different characteristics in the orbicularis oculi muscle using stimuli of other modality. For example, the cutaneous R1 reflex evoked by supraorbital electrical stimulation is mediated by a pontine pathway without involving the reticular formation and shows an inverse modification in many paradigms in comparison to the later components (R2,R3) that are believed to belong to the startle reaction in man [7, 18–20]. Also after visual stimulation two overlapping reflexes with different physiological traits can be elicited, but only the R 50 component is thought to be part of the startle pattern [5].

In contrast to previous work [21], in the present study prestimulations were found to have a stronger inhibitory effect on the startle in comparison to the blink reflex: Whereas in pulse-alone trials the amplitude of the first peak was found to be considerably smaller than that of the second, in prepulse-pulse trials they show on average similar amplitudes. Furthermore, prepulse inhibition of the second peak was more marked than prepulse inhibition of the first peak, i. e., the auditory blink reflex. In addition, the offset latency facilitation of blink responses was more pronounced in the PP100 trials and considerably stronger than the facilitation of the onset latency. This finding may be interpreted as a more marked shortening of later reflex portions indicating a relatively stronger inhibition of the prolonged startle reflex which has been found to be grafted onto the end of the auditory blink reflex [3].

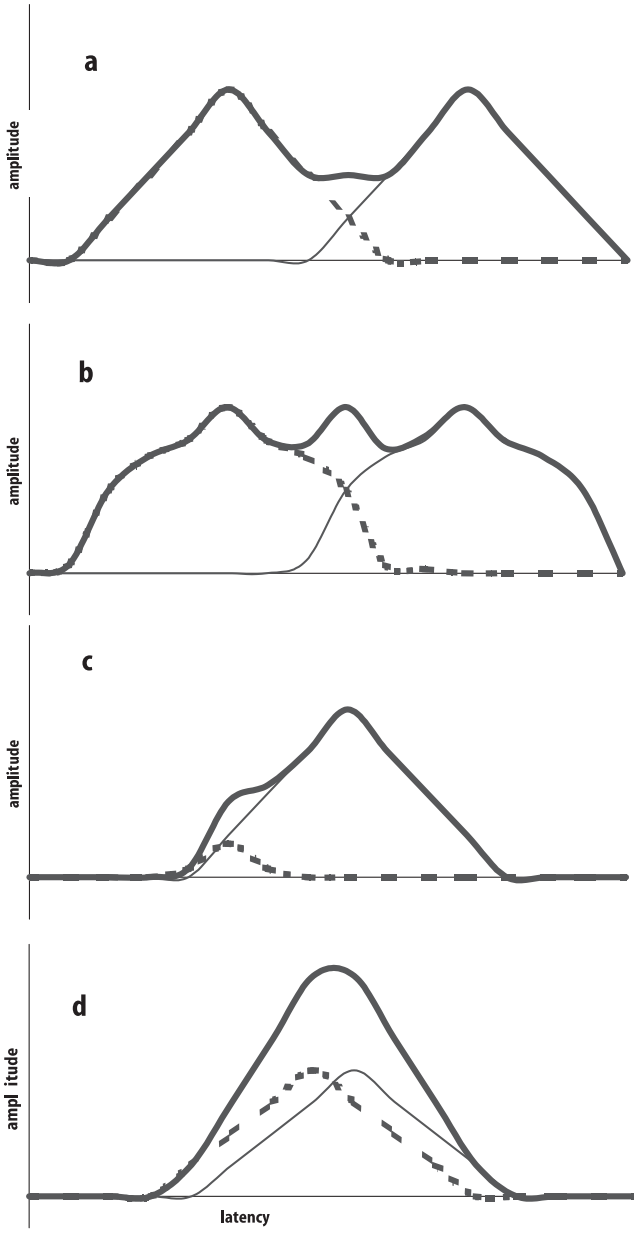

Fig. 3 a–d Schematic illustration of possible summations of the auditory blink reflex (dotted line) and the startle reflex (fine line) resulting in the blink response recorded electromyographically (solid line).

Our result of a differential prepulse inhibition is not in agreement with the study of Valls-Solé et al. [21], who found similar prepulse inhibition of both components. This discrepancy might be explained by methodological differences, i.e., different stimulus intensities. However, our finding of differential modulation of startle and blink reflexes is in line with the idea that auditory blink and startle reflexes are distinct electromyographical phenomena [3] reflecting different physiological functions. In addition, it has been suggested that they are mediated by different neuroanatomical pathways: for the auditory blink reflex the inferior colliculi and the midbrain reticular formation seem to be critical structures

[3], whereas the bulbopontine arc of the startle reflex involves the cochlear root neurons and the nucleus reticularis pontis caudalis [6, 12, 20].

An additional argument that the early auditory blink reflex might be more resistant to prepulse inhibition than the later startle reflex comes from studies investigating electrocutaneous and photic blink responses. It has been shown that the R2 component of the electrically elicited blink reflex, which is also the electromyographical correlate of the visible eyelid closure, is suppressed predominately in the later sections in paradigms of habituation, prepulse inhibition [8] and self-elicitation [13, 15]. Furthermore, the early portion of the visually evoked R 50 component is stable to the inhibitory influence of prepulses [10].

Also it is known that the human auditory blink reflex and the acoustic startle reflex may show dissociated modulation in pathological conditions, such as the hereditary startle disease [4], that may reflect different physiological traits of the auditory blink and the acoustic startle reflex. It will be the subject of further research to investigate comparatively PPI of blink and startle reflexes in other psychiatric disorders, such as in patients with schizophrenia.

In conclusion, in blink responses with two peaks the later component with a peak latency of about 50 ms and above has to be measured in studies investigating the modulation of the startle reflex in humans. However, in many responses after 115 dB pulses it is not possible to distinguish between startle and blink reflexes, because these reflexes extensively overlap resulting in a waveform with one peak. We propose that the differentiation of these distinct components in double-peaked auditory blink responses should be emphasized in further research in order to avoid misleading interpretations in studies investigating startle behavior.

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