**Abstract** Recent evidence suggests that electromyo-
graphic 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

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**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 kΩ.

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.