



Lateralization effects on the cardiac modulation of acoustic startle eye blink

André Schulz^{a,*}, Johanna Lass-Hennemann^a, Steffen Richter^a, Sonja Römer^a,
Terry D. Blumenthal^b, Hartmut Schächinger^a

^a Division of Clinical Physiology, Institute of Psychobiology, University of Trier, Johanniterufer 15, D-54290 Trier, Germany

^b Department of Psychology, Wake Forest University, Winston-Salem, NC, USA

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ABSTRACT

Cardiac modulation of startle eye blink has been introduced as a methodology to reflect baro-afferent signal transmission. Recent studies showed that affective startle modulation is specific to left-ear presentation that may be due to hemispheric specificity in processing emotional-relevant stimuli, similar to the processing of visceral- and baro-afferent stimuli. To explore whether cardiac modulation of startle eye blink is lateralized as well, 37 healthy volunteers received 160 unilateral acoustic startle probes of 105 dB(A) intensity presented to both ears, one at a time. They were elicited 0, 100, 230, and 530 ms after the R-wave of the cardiac cycle. Startle response magnitude was significantly diminished at a latency of 230 ms, which may be due to the baro-afferent neural feedback at this temporal location, but only for left-ear presentation. This lateralization effect in the cardiac modulation of startle eye blink may reflect the previously described advantages of right-hemispheric brain structures in relaying viscer- and baro-afferent signal transmission.

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1. Introduction

Perception of bodily states plays an important role in the subjective experience of emotion (Wiens, 2005), consciousness (Damasio, 2003), and symptom genesis (Eley et al., 2004). The current methodological repertoire to assess interoception involves heartbeat perception paradigms that depend on participants' active cooperation, such as heartbeat counting (Schandry, 1981) and heartbeat discrimination tasks (Whitehead et al., 1977). Periodically increased feedback from the arterial baroreceptors, which are also responsible for cardiac perception (Dworkin, 2000), is known to impact simple cognitive functions, such as prolonged reaction times (Edwards et al., 2007), and brainstem-relayed reflexes, such as startle responses (Nyklicek et al., 2005; Schulz et al., 2008). Thus, the cardiac modulation of startle eye blink has been introduced to assess baro-afferent neural feedback, with the pre-attentive startle response being independent of participants' active cooperation, since it relies on intact baro-afferences and does not depend on conscious processing of cardiovascular signals (Schulz et al., 2008).

It is well described that central processing of active heartbeat perception (Critchley et al., 2004; Pollatos et al., 2007) as well as 'background' stimulation of baroreceptors (Henderson et al., 2004; Kimmerly et al., 2005) involves similar subcortical and cortical brain regions. For example, on the brainstem level, the nucleus tractus solitarius and, on the cortical level, the anterior insula, play a crucial role in relaying cardiac-afferent information. However, higher cortical brain regions that are involved in relaying viscer- and baro-afferent signals have been found to be lateralized, i.e., to have advantages in responsiveness within the right hemisphere (Critchley et al., 2004; Henderson et al., 2004; Weisz et al., 2001). Furthermore, increased performance of visceral perception was found when right-hemispheric cognitive functions were activated (Katkin and Reed, 1988; Weisz et al., 1994). It can be concluded that processing of interoceptive neural signals may be specific to right-hemispheric brain structures.

Thus, we aimed to discover whether the cardiac modulation effect of acoustically elicited startle responses may be specific to lateral stimulation presented to either the left or the right ear. Earlier results indicate that startle responsiveness is a lateralized phenomenon. Unilateral presentation of acoustic startle probes results in greater startle eye blink response EMG (electromyographic) magnitude at the ipsilateral orbicularis oculi (Bradley et al., 1991; Bradley et al., 1996; Hackley and Graham, 1987; Kettle et al., 2006; Kofler et al., 2008), whereas generally facilitated

* Corresponding author. Tel.: +49 651 201 3735/2164; fax: +49 651 201 3737.
E-mail address: andre.schulz@uni-trier.de (A. Schulz).

responsiveness with right-ear stimulation is less consistently found (Hackley and Graham, 1987; Kofler et al., 2008). It has also been shown that affective startle modulation is specific to left-ear stimulation (Bradley et al., 1991; Bradley et al., 1996; Kettle et al., 2006), while right-ear presentation produces no or inconsistent affective modulation. Given that processing of affective stimuli also has advantages in right-hemispheric brain structures (Carmon and Nachshon, 1973; Haggard and Parkinson, 1971; Joseph, 1988), it may be concluded that affective startle modulation is mainly processed by right-hemispheric brain structures, although lesion studies have shown discrepant findings on this issue (Funayama et al., 2001; Kettle et al., 2006).

To test this lateralization hypothesis for the cardiac modulation of startle eye blink, we conducted a within-subjects experiment. Thirty-seven healthy volunteers received 160 randomized startle probes of 105 dB(A) intensity that were elicited with latencies of 0, 100, 230, and 530 ms after an R-wave of the cardiac cycle. We extended the original study protocol (Schulz et al., 2008) by the two additional latencies of R +0 ms and R +100 ms to explore whether the baro-afferent modulation effect is also present at these early latencies. We hypothesized that the maximal baroreceptor stimulation during the latency of R +230 ms would cause a diminished startle response magnitude in this condition, and that this effect would only be present in left ear startle stimulation. This may identify possible neural pathways that underlie the cardiac modulation of acoustic startle effect.

2. Methods

2.1. Participants

Forty-four healthy right-handed undergraduate students (28 females) participated to receive course credit. Physical health status was assessed by a health questionnaire. Hearing problems (impairments, tinnitus), regular use of contact lenses, any actual health complaints, abuse of illicit drugs within the last 6 months, medication other than occasional pain killers and oral contraceptives, or confirmed somatic or psychiatric diseases within the last six months other than banal infections or minor injuries, were exclusion criteria. Furthermore, we excluded participants with a resting heart rate of higher than 85 bpm to prevent startle stimuli from extending into the following cardiac cycle. Mean age of the participants was 23.8 (range: 20–29; S.D. = 2.5) years. Mean heart rate was 76.6 (S.D. = 11.8) bpm. All participants provided written informed consent and were made aware of their right to discontinue participation in the study at any time. Seven participants were excluded from analysis because of strong habituation and absence of any visible eyeblink responses during the experimental condition, technical malfunctions, or a resting heart rate of higher than 85 bpm. The remaining thirty-seven participants (25 women) had a mean age of 23.6 (S.D. = 2.4) years and a mean resting heart rate of 70.5 (S.D. = 8.8) bpm. All participants had a regular sinusoidal cardiac rhythm without premature heartbeats. Study procedures were approved by the local ethical committee.

2.2. Procedure

Participants were seated in front of a LCD computer display in a comfortable chair. Electrodes for ECG-measurement (ECG Tyco Healthcare H34SG Ag/AgCl electrodes of 45 mm diameter) were placed according to a standard lead II configuration. Glasses were removed and pairs of Tyco Healthcare H124SG electrodes (diameter: 24 mm) were attached below each eye with an inter-electrode distance of 1.5 cm to assess EMG-activity of the orbicularis oculi. Headphones (Sennheiser electronic GmbH & Co. KG, Wedemark, Germany) were attached. Participants were informed about the experimental procedures on the computer display. They were asked to relax, neither speak nor move, avoid longer periods of eye closure, and listen carefully to all acoustic stimuli.

At the beginning of the experimental session six startle probes without any contingency to the participants' heartbeat served as habituation trials. These EMG-responses were not further analyzed. During the experimental procedure startle probes were presented with a jittering inter-stimulus-interval of 8–12 s, according to a completely randomized sequence over all experimental conditions: startle stimuli appeared randomly with one of four latencies (0, 100, 230, or 530 ms) after the detection of an ECG-recorded R-wave, and were presented to the left or to the right ear. In each of these $4 \times 2 = 8$ conditions, 20 stimuli were presented, 160 stimuli overall. Total length of the experimental session, including instructions, was about 45 min.

2.3. Recording parameters

EMG-responses of the left and the right orbicularis oculi to acoustic white noise startle probes (105 dB, 50 ms duration, instantaneous rise time, monaural stimulation) were recorded on hard disk with a Biopac MP150 system at 16 bit resolution and 1 kHz sampling rate. Hardware band-pass filter settings (Nihon Kohden EEG 4421G pre-amplifier) were 0.5–500 Hz, followed by software filtering (28 Hz high pass cutoff: van Boxtel et al. (1998)). The raw signals of both sides were rectified and integrated online with a time constant of 10 ms (Blumenthal, 1994). The ECG signal was high-pass filtered (Biopac ECG100C, HPF: 0.5 Hz) and stored to disk (1 kHz) as well. R-waves were identified online by a fast DASLAB-8.0 (National Instruments, Inc.) algorithm running on a different CPU, based on a gradient detection, the threshold of which was set separately for each participant. Accuracy of R-wave detection in sine rhythm was higher than 99.8%, with a latency below 3 ms (internal lab report).

2.4. Startle response analysis

A customized C++ based semi-automated PC program was used on a WinXP platform to analyze EMG responses offline. The algorithm identified response peaks in the rectified and integrated signal in the time interval of 20–150 ms after the startle probe onset. The baseline period was defined by a 50 ms interval prior to acoustic stimulation. All response data were manually confirmed. Signals with electrical and physiological artifacts, such as coinciding blinks or other facial muscular activities, were rejected from analysis and defined as missing. The mean artifact rate per subject was 7.61% (S.D. = 7.67%). If responses were not visible at the typical response latency of a particular subject, or if the response amplitude was below a threshold of 10 μ V, response amplitude was set to zero. Zero response data were included in the averaging procedure, with startle response magnitude as the final output measure (Blumenthal et al., 2005). Averaging was done per subject and according to the experimental condition (side of presentation/side of assessment/cardiac cycle).

2.5. Statistical analysis

A $2 \times 2 \times 4$ repeated-measurement ANOVA was employed with the within-subjects factors (i) side of presentation (left/right), (ii) side of EMG measurement (left/right) and (iii) cardiac cycle phase (R-wave +0 ms/100 ms/230 ms/530 ms). Critical alpha-level was set to 0.05. All effects originating from repeated measurements with more than two conditions are reported with Greenhouse-Geisser corrections. For post-hoc analysis of differences between conditions, dependent *t*-tests were calculated. The dependent variable of the ANOVA model was the startle response magnitude.

3. Results

Our analysis within the ANOVA model indicated no significant main effect for the presentation side (left: $M = 111 \mu$ V; SEM = 21 μ V; right: $M = 113 \mu$ V; SEM = 21 μ V; $F[1,36] < 1$), nor for the side of measurement (left: $M = 111 \mu$ V; SEM = 23 μ V; right: $M = 114 \mu$ V; SEM = 21 μ V; $F[1,36] < 1$). However, the interaction of presentation side \times side of measurement was significant ($F[1,36] = 12.556$; $p < .001$; $\eta^2 = .259$), in that startle response magnitude was larger in ipsilateral presentation (left: $M = 122 \mu$ V; SEM = 25 μ V; right: $M = 126 \mu$ V; SEM = 23 μ V) than in contralateral presentation (left: $M = 101 \mu$ V; SEM = 19 μ V; right: $M = 101 \mu$ V; SEM = 20 μ V) (see Fig. 1a and b).

Replicating recent results (Schulz et al., 2008), the main effect of cardiac cycle was significant ($F[3,108] = 4.433$; $p < .025$; $\eta^2 = .110$), indicating that startle was inhibited when startle stimuli were presented with a latency of 230 ms after the R-wave ($M = 105 \mu$ V; SEM = 20 μ V), compared to the latencies of 0 ms ($M = 115 \mu$ V; SEM = 22 μ V), 100 ms ($M = 111 \mu$ V; SEM = 21 μ V), and 530 ms ($M = 117 \mu$ V; SEM = 22 μ V). Post-hoc analyses revealed that reactivity in the 230 ms latency condition differed significantly from that in all other latency conditions (0 ms vs. 230 ms: $p < .001$; 100 ms vs. 230 ms: $p < .05$; 530 ms vs. 230 ms: $p < .025$). Thus, the effect of cardiac modulation of startle eye blink is specific to the 230 ms latency condition and does not appear with shorter latencies to the R-wave during the cardiac systole, nor with the longer latency of 530 ms.

Furthermore, we observed a significant interaction of presentation side \times cardiac cycle ($F[3,108] = 4.609$; $p < .01$; $\eta^2 = .113$).

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