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Cardiac Modulation of Startle Eye Blink: A Pre-Attentive Method to Assess Interoceptive and Baro-Afferent Neural Traffic.

Autor:

André Schulz, Dipl.-Psych.

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Gutachter:

Prof. Dr. med. Hartmut Schächinger

Dr. rer. nat. Ewald Naumann

This dissertation thesis and the presented research were performed at the

Division of Clinical Physiology

Institute of Psychobiology – University of Trier.

Affiliation of the Supervisors:

Prof. Dr. med. Hartmut Schächinger

Division of Clinical Physiology – Institute of Psychobiology – University of Trier

Dr. rer. nat. Ewald Naumann

Psychophysiological Laboratory – Department of Psychology – University of Trier

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General Abstract (English)

Interoception – the perception of bodily processes – plays a crucial role in the subjective experience of emotion, consciousness and symptom genesis. As an alternative to interoceptive paradigms that depend on the participants' active cooperation, five studies are presented to show that startle methodology may be employed to study visceral afferent processing. *Study 1* (38 volunteers) showed that startle responses to acoustic stimuli of 105 dB(A) intensity were smaller when elicited during the cardiac systole (R-wave +230 ms) as compared to the diastole (R +530 ms). In *Study 2*, 31 diabetic patients were divided into two groups with normal or diminished (< 6 ms/mmHg) baroreflex sensitivity (BRS) of heart rate control. Patients with normal BRS showed a startle inhibition during the cardiac systole as was found for healthy volunteers. Diabetic patients with diminished BRS did not show this pattern. Because diminished BRS is an indicator of impaired baro-afferent signal transmission, we concluded that cardiac modulation of startle is associated with intact arterial baro-afferent feedback. Thus, pre-attentive startle methodology is feasible to study visceral afferent processing.

Visceral- and baro-afferent information has been found to be mainly processed in the right hemisphere. To explore whether cardiac modulation of startle eye blink is lateralized as well, in *Study 3*, 37 healthy volunteers received 160 unilateral acoustic startle stimuli presented to both ears, one at a time (R +0, 100, 230, 530 ms). Startle response magnitude was only diminished at R +230 ms and 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 viscero- and baro-afferent signal transmission.

This lateralization effect implies that higher cognitive processes may also play a role in the cardiac modulation of startle. To address this question, *in Study 4*, 25 volunteers responded first by 'fast as possible' button pushes (reaction time, RT), and second, rated perceived intensity of 60 acoustic startle stimuli (85, 95, or 105 dB; R +230, 530 ms). RT was divided into evaluation and motor response time. Increasing stimulus intensity enhanced startle eye blink, intensity ratings, and RT components. Eye blinks and intensity judgments were lower when startle was elicited at a latency of R +230 ms, but RT components were differentially affected. It is concluded that the cardiac cycle affects the attentive processing of acoustic startle stimuli.

Beside the arterial baroreceptors, the cardiopulmonary baroreceptors represent another important system of cardiovascular perception that may have similar effects on startle responsiveness. To clarify this issue, *in Study 5*, Lower Body Negative Pressure at gradients of 0, -10, -20, and -30 mmHg was applied to unload cardiopulmonary baroreceptors in 12 healthy males, while acoustic startle stimuli were presented (R +230, 530 ms). Unloading of cardiopulmonary baroreceptors increased startle eye blink responsiveness. Furthermore, the effect of relative loading/unloading of arterial baroreceptors on startle eye blink responsiveness was replicated. These results demonstrate that the loading status of cardiopulmonary baroreceptors also has an impact on brainstem-based CNS processes.

Thus, the cardiac modulation of acoustic startle is feasible to reflect baro-afferent signal transmission of multiple neural sources, it represents a pre-attentive method that is independent of active cooperation, but its modulatory effects also reach higher cognitive, attentive processes.

Zusammenfassung (deutsch)

Interozeption, die Wahrnehmung von Körpersignalen, spielt eine wichtige Rolle bei subjektiver Empfindung von Emotionen, Bewusstsein und Symptomgenese. Als Alternative zu Interozeptionsparadigmen, die von der aktiven Mitarbeit der Probanden abhängen, werden hier insgesamt fünf Studien vorgestellt, mit denen die Anwendbarkeit der Schreckreaktionsmodulation für die Messung viszeral-afferenter Signale belegt wird. *Studie 1* mit 38 gesunden Probanden zeigte, dass Schreckreaktionen auf akustische Stimuli mit 105 dB(A) Intensität geringer sind, wenn sie in der kardialen Systole (R-Zacke +230 ms) im Vergleich zur Diastole (R +530 ms) ausgelöst werden. In *Studie 2* wurden 31 Diabetespatienten in zwei Gruppen geteilt, die entweder eine normale oder verringerte (< 6 ms/mmHg) Baroreflex-Sensitivität (BRS) der Herzratenkontrolle aufwiesen. Die Patienten mit normaler BRS zeigten die gleichen Ergebnisse wie die gesunden Probanden, während die Patienten mit verringerter BRS dieses Modulationsmuster nicht zeigten. Da verringerte BRS ein Indikator für beeinträchtigte baro-afferente Signalübermittlung darstellt, könnte geschlussfolgert werden, dass ein kardialer Modulationseffekt der Schreckreaktion mit intakter arterieller baro-afferenter Rückmeldung assoziiert ist. Daher ist die Schreckreaktions-Methodik geeignet, um viszeral-afferente Signalverarbeitung abzubilden.

Es wurde mehrfach gezeigt, dass baro-afferente Signalverarbeitung rechtshemisphärische Verarbeitungsvorteile aufweisen. Um zu überprüfen, ob der kardiale Modulationseffekt der Schreckreaktion auch einer Lateralisierung unterliegt, wurden in *Studie 3* 37 gesunden Probanden je 80 monaurale Schreckreize pro Ohr präsentiert (R +0, 100, 230, 530 ms). Die Schreckreaktion war nur bei R +230 ms und linksseitiger Stimulation verringert. Dieser Lateralisierungseffekt der kardialen Modulation der Schreckreaktion könnte mit den rechtshemisphärischen Vorteilen in der Verschaltung von viszeral- und baro-afferenten Signalen zusammenhängen.

Der gefundene Lateralisierungseffekt könnte bedeuten, dass auch höhere kognitive Prozesse bei der kardialen Modulation der Schreckreaktion eine Rolle spielen. Um diese Annahme zu überprüfen, sollten in *Studie 4* 25 gesunde Probanden auf 60 Schreckreize (85, 95, 105 dB(A); R +230, 530 ms) so schnell wie möglich mit Knopfdruck reagieren und danach deren subjektive Intensität einschätzen. Die Reaktionszeit (RT) wurde in Verarbeitungs- und Bewegungszeit differenziert. Höhere Stimulusintensität führte zu stärkeren Schreckreaktionen, höherer subjektiver Intensität und schnelleren RT-Komponenten. Die

Schreckreaktionen und subjektiven Intensitäten waren geringer bei R +230 ms, während die RT-Komponenten durch die kardialen Phasen entgegengesetzt moduliert wurden. Dies spricht dafür, dass der kardiale Zyklus auch die attentive Verarbeitung von Schreckreizen moduliert.

Neben arteriellen Barorezeptoren stellen die kardiopulmonaren Barorezeptoren ein weiteres System kardiovaskulärer Wahrnehmung dar, das ebenso einen Effekt auf Schreckreaktionen haben könnte. Zur Klärung dieser Frage wurde in *Studie 5* 12 gesunden Männern abdominaler Unterdruck von 0, -10, -20 und -30 mmHg appliziert, um eine Disstimulation kardiopulmonarer Barorezeptoren zu bewirken, während akustische Schreckreize präsentiert wurden (R +230, 530 ms). Eine Disstimulation der kardiopulmonaren Barorezeptoren führte zu einer Verstärkung der Schreckreaktion. Weiterhin wurde der Effekt der relativen Stimulation der arteriellen Barorezeptoren auf die Schreckreaktion repliziert. Diese Ergebnisse zeigen, dass die Stimulation kardiopulmonarer Barorezeptoren ebenfalls einen Einfluss auf zentrale Prozesse auf Hirnstammebene hat.

Zusammenfassend ist die kardiale Modulation der Schreckreaktion geeignet, um baro-afferente Signalübermittlung aus unterschiedlichen Quellen abzubilden. Sie ist eine präattentive Methode, die ohne die aktive Mitarbeit von Probanden auskommt, obwohl ihre Modulationseffekte ebenfalls höhere kognitiv-attentive Prozesse beeinflussen.

Content

Index of Figures

Index of Tables

Index of Publications

This doctoral thesis consists in general of four chapters (and one additional chapter that represents a general introduction and overview), which are published as 'Original Research Articles' in international peer-reviewed journals. All articles are presented here in the originally published form, except for changes in formatting (i.e. figure labeling, references).

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Index of Abbreviations

Ag/AgCl	Silver/Silver chloride
AI	Anterior insula
ANOVA	Analysis of variance
ANS	Autonomic nervous system
AU	Arbitrary units
bpm	Beats per minute
BMI	Body mass index
BRS	Baroreflex sensitivity
CMS	Cardiac modulation of startle
CNS	Central nervous system
CNV	Contingent negative variation
CPU	Central processing unit
CVLM	Caudal ventrolateral medulla
dB(A)	Decibels (A-scale)
dl	Deciliters
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyogram
EVAS	Electronic Visual Analog Scale
FFT	Fast Fourier Transformation
HbA_{1c}	Glycosylated haemoglobine
HEP	Heartbeat-evoked potential
HF	High frequency
HPF	High-pass filter
Hz	Hertz
ICG	Impedance cardiography
ISI	Inter-stimulus interval
LBNP	Lower body negative pressure
LC	Locus coeruleus
LCD	Liquid crystal display
LF	Low frequency
M	Mean
M.	Musculus
mg	Milligrams
min	Minutes
ml	Milliliters
mmHg	Millimeters mercury
ms	Milliseconds
MT	Movement time
mV	Millivolts

μV	Microvolts
NA	Nucleus ambiguus
NRPC	Nucleus reticularis pontis caudalis
NTS	Nucleus tractus solitarius
NU	Normalized units
PPI	Prepulse inhibition
PRES	Phase related external suction
RT	Reaction time
RVLM	Rostral ventrolateral medulla
s	Seconds
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
SEM	Standard error of mean

Chapter I:

General Rationale

The aim of the present work is to establish a new methodology to describe the impact of visceral afferent neural traffic on pre-attentive basic cognitive processes. The perception of bodily states via visceral afferents plays a crucial role in various psychological concepts. Visceral information is integrated in the subjective experience of emotion (Craig, 2002; Wiens, 2005). In the original James-Lange theory, the genesis of an emotion was hypothesized to be a central representation of sensations originating from the body (James, 1994). Although this perspective may be outdated from a current point of view, neuroscientific results confirm the existence of pathways relaying visceral information and shaping emotional states (Critchley, Wiens, Rotshtein, Ohman & Dolan, 2004). Today, it is a broadly accepted thesis that visceral perceptions contribute to various cognitive processes. For instance, Damasio's somatic marker theory postulates that internal body information is involved in self-consciousness (Damasio, 2003). From a clinical point of view, the perception of body functions is considered to be essential for symptom genesis in body-related psychiatric disorders. Accordingly, altered interoceptive awareness and/or accuracy was found in patients with panic (Ehlers & Breuer, 1992; Ehlers & Breuer, 1996; Ehlers, Breuer, Dohn & Fiegenbaum, 1995) and somatoform disorders (Barsky, Brener, Coeytaux & Cleary, 1995; Mussgay, Klinkenberg & Ruddel, 1999). The importance of body perception in clinical psychology is even more emphasized in models proposing overlapping brain structures for interoceptive and pain processing (Craig, 2002).

1.1 Current Research on Interoception

The current methods for exploring interoception in humans focus on self-reported sensations originating from the cardiovascular system that are compared with objectively measurable values. Firstly, in the heartbeat-counting tasks (Schandry, 1981a), participants are asked to count their heartbeats in a defined interval. The number of subjectively perceived heartbeats is then compared to the objectively measured number of heartbeats. Secondly, in the exteroceptive-interoceptive discrimination tasks, exteroceptive stimuli (for example tones) are presented either simultaneously or with a short delay to the participants' heartbeats.

Participants are asked to indicate whether the tones were simultaneous or delayed to their heartbeats (Whitehead, Drescher, Heiman & Blackwell, 1977). Although performance scores in the heartbeat-counting and the heartbeat-discrimination paradigms are positively correlated (Knoll & Hodapp, 1992), the validity of both methods have been questioned. The heartbeat-counting task has been criticized, because it is thought to measure the participants' ability to estimate time intervals instead of measuring their interoceptive performance. The main critique on the heartbeat-discrimination paradigm is the high degree of difficulty in performing the task, which may produce unwanted variance in an experimental setting because required attentional resources may be captured by the experimental task. These may be two reasons for the conflicting results regarding the impact of autonomic activation on interoceptive accuracy in the heartbeat-discrimination and the heartbeat-counting task (Fairclough & Goodwin, 2007; Schandry, Bestler & Montoya, 1993). Thus, it remains unclear whether autonomic arousal and stress, which contribute to the genesis of various psychiatric and psychosomatic disorders, modulate one's capabilities in perceiving body-related signals. From this point of view, the development of a method that is independent from task difficulty and involvement of attentional resources of the participating individuals would be of great value.

1.2 Methodological Considerations

Discrepant findings in the interoceptive accuracy of psychiatric patients are often attributed to methodological issues that are related to the participants' active cooperation and motivation. For instance, interoceptive accuracy scores are strongly sensitive to the previously given instructions (Ehlers et al., 1995). Furthermore, subjective reports of interoceptive awareness often differ from performance scores assessed by the common heartbeat detection paradigms (Khalsa et al., 2008). Systematic assessment of subjective body perception via questionnaires also reveals a lack of cross-methodological correlation. Questionnaires on interoceptive awareness either focus on clinical aspects or on the (re-) activity of the autonomic nervous system. A well described clinical instrument is the 'Somatosensory Amplification Scale (SSAS)' (Barsky & Wyshak, 1990; Barsky, Wyshak & Klerman, 1990), which primarily assesses the patients' awareness of potential symptoms of physical illness. Past studies have shown that there is no association between SSAS- Scores as indicators for the severeness of somatoform disorders and interoceptive awareness measured by heartbeat-perception paradigms (Barsky et al., 1995). The 'Body Perception Questionnaire' also lacks evidence for

its validity (BPQ) (Porges, 1993). Although the BPQ has different subscales for the perceived reactivity of the autonomic nervous system and individual stress responses which may in parts correlate with heartbeat perception paradigms (Fairclough & Goodwin, 2007), it does not provide any subjective score for body perception during rest. Thus, experimental heartbeat paradigms and variants of body perception questionnaires may all assess different aspects of interoception and therefore address different parts of the statistical variance of the psychobiological construct of interoceptive accuracy. This may explain the poor to moderate correlations between these measures. Until now, there is no method available for quantifying actual neural traffic from the body to the brain or central processing mechanisms of viscero-afferent neural traffic. In the present work I will describe a method for interoceptive assessment, which has been designed to overcome the limitations of the previously described interoceptive paradigms. This newly developed method is independent of the participants' active cooperation and focuses on physiology-based mechanisms of baro-afferent neural traffic.

1.3 Arterial and Cardiopulmonary Baroreceptors

It is assumed that arterial baroreceptors are mainly responsible for the perception of cardiovascular activity (Cameron, 2002; Dworkin, 2000). Thus, it is reasonable to attempt measuring baro-afferent neural traffic independently of participants' active involvement. There are various paradigms to stimulate arterial baroreceptors mechanically (Rau et al., 1994; Rau, Elbert, Geiger & Lutzenberger, 1992) or pharmacologically (Schachinger, Weinbacher, Kiss, Ritz & Langewitz, 2001). However, these methods are either invasive, unecological or uncomfortable for the participants, or fail to assess baro-afferent neural traffic. For example, the non-invasive measurement of beat-to-beat data of heart rate and blood pressure allows for an estimation of baroreflex-sensitivity (BRS) of heart rate control based on the coherence of variation in both signals (Robbe et al., 1987) (throughout this work, 'baroreflex-sensitivity of heart rate control' will be referred to with the abbreviation 'BRS'). The baroreflex guarantees for a homeostatic regulation of heart rate and blood pressure in healthy subjects (Jänig, 2006). A diminished BRS is considered to be an early indicator of impaired autonomic neural traffic (Frattola et al., 1997), but it does not differentiate between baroreflex afferents (from baroreceptors to sensory centers in the brainstem) and efferents (from motor centers in the brainstem to cardiac effector cells). However, only afferent neural traffic is important for interoceptive accuracy. Thus, it is reasonable to develop a

methodology focusing on the non-invasive measurement of afferent neurotransmission from the cardiovascular system to the brain. Since different impacts of baroreceptor loading on CNS functions are known, one strategy may be the indirect measurement of baro-afferent neural feedback by estimating these baro-afferent modulatory effects on the CNS. Hence, different modulatory effects of baroreceptors on CNS functions and responsible neural pathways need to be evaluated to determine a useful indicator of the amount of baro-afferent neural feedback.

1.3.1 Physiology of Baroreceptors

Baroreceptors are stretch receptors that are located in the blood vessels. These stretch receptors respond to changes in the pressure within the blood vessels and, besides the chemoreceptors, represent one of the two major sources of cardiovascular sensory information (Jänig, 2006; Milnor, 1990). Stimulation of baroreceptors results in a relaying of information at the brainstem level, although afferent neural traffic also reaches higher CNS structures and produces effects of homeostatic blood pressure regulation. These effects are that higher blood pressure leads to a decrease, whereas lower blood pressure leads to an increase in heart rate to ensure an appropriate activation level of the cardiovascular system. In general, the baroreceptor system can be divided into the 'high pressure' and the 'low pressure' system. The 'high pressure' system consists of baroreceptors in the wall of the arterial blood vessels, especially in the wall of the aortic brach and the carotis. These 'arterial' baroreceptors respond rapidly to changes in the filling of these blood vessels. These changes can either be a static increase of blood pressure or phasic variations of blood pressure elicited by the arterial pulse wave within the cardiac cycle. Arterial baroreceptor information is transmitted via myelinated A-type or unmyelinated C-type fibers (Coleridge, Coleridge & Schultz, 1987). The main differences between both are the firing thresholds, the firing frequency, the adaptation time and the velocity of action potentials. Nevertheless, no differences in synaptic pathways, reflex regulatory effects, or CNS effects are known. Thus, the presence of two arterial baroreceptor afferent fiber types may only reflect a distinction in their operating range of the blood pressure level, but not a functional difference (Dembowski & Seller, 1995). The 'low pressure' or 'cardiopulmonary' baroreceptor system responds to changes in the filling status of the venous system and the cardiac atria, and is therefore sensitive to much lower variations in the blood pressure. However, this system has a longer response latency and adaptivity (Jänig, 2006) and therefore does not respond to blood pressure changes within the

cardiac cycle. For instance, a decreased total availability of blood due to a critical blood loss may induce a compensatory increase of heart rate to ensure an appropriate blood supply.

The neural information of both baroreceptor systems reaches the first synapse in the Nucleus tractus solitarius (NTS), which is a major brainstem center for the relaying of visceral-afferent signal transmission. From the NTS, the parasympathetic efferent branch of the arterial baroreceptor reflex circuit innervates the Nucleus ambiguus (NA), which directly impacts on the cardiac pacemaker. The sympathetic branch projects over the Caudal ventrolateral medulla (CVLM) onto the Rostral ventrolateral medulla (RVLM) (Jänig, 2006). From there, cardiomotor and vasomotor centers may be stimulated. Beyond those brainstem-based reflex pathways, neural information from the NTS also reaches higher brain structures. The NTS is directly and indirectly (via the Parabrachial nucleus) connected with the Limbic system and the Thalamus, which project onto the Insular cortex (Dembowski & Seller, 1995). Although the reflex pathways of both baroreceptor systems are simple neural circuits that involve only a small number of synapses, different types of baroreceptors exist that all have similar, but not identical neural pathways. For instance, cardiopulmonary baroreceptor information is also relayed over the RVLM and affects cardiomotor and vasomotor activity, but there is one synapse between the NTS and the RVLM that differs (Jänig, 2006). It is important to note that the stimulation of both baroreceptor types result in an activation of the Insula (Henderson et al., 2004; Kimmerly, O'Leary, Menon, Gati & Shoemaker, 2005), which represents an important cortical structure for visceral-afferent information processing. Furthermore, the performance scores in heartbeat-counting and heartbeat-discrimination paradigms are correlated with activity and gray matter substance of the Insula (Critchley et al., 2004; Pollatos, Gramann & Schandry, 2007a; Pollatos, Kirsch & Schandry, 2005; Pollatos, Schandry, Auer & Kaufmann, 2007b). It is conceiveable that baro-afferent signal transmission may be the crucial neural substrate of cardiac perception, although the distinct role of the different baroreceptor systems and possible interactions remain unclear. However, the current work focuses on the non-invasive measurement of this baro-afferent neural traffic.

1.3.2 Lacey's Hypothesis

An influential theory in the field of baroreceptor-brain interaction is Lacey's Baroreceptor Hypothesis (Lacey & Lacey, 1974; Lacey & Lacey, 1978; Lacey, 1967). Lacey and colleagues postulated that afferent neural signal transmission, originating from baroreceptors, modulates higher brain functions. Birren and colleagues first showed an inhibition of psychomotor responses during the early cardiac cycle phase (Birren, Cardon & Phillips, 1963). The authors concluded that the pulsatile stimulation of arterial baroreceptors by the arterial pulse wave leads to an inhibition of sensorimotor function, such as stimulus perception and the elicitation of a motor response. Although the actual pathway and functionality of potentially inhibiting baro-afferent feedback remains unclear, many different inhibitory effects of central baroreceptor impact have been described in the literature. Newer studies have emphasized the responsibility of the arterial pulse wave for the modulatory effects on psychomotor response times: A prolongation in psychomotor responding only appears between a latency of 200 to 400 ms after the R-wave, while the occurrence of the arterial pulse wave is expected (Edwards, Ring, McIntyre, Carroll & Martin, 2007; Stewart, France & Suhr, 2006).

This association has a clinical relevance, because beyond the effects of phasic baroreceptor loading across the cardiac cycle, a tonic increase of baroreceptor loading was also found to cause comparable effects on simple cognitive functions. For instance, chronically hypertensive patients show deficits in cognitive tests that assess simple psychomotor response times (Blumenthal, Madden, Pierce, Siegel & Appelbaum, 1993; Harrington, Saxby, McKeith, Wesnes & Ford, 2000; Karla, Jackson & Swift, 1993). Although there is much evidence for a modulatory effect of baro-afferent neural traffic on CNS processes, few studies have addressed the question whether the central processing of the stimulus or the motor response to the stimulus is inhibited by baroreceptor feedback. Only one study decomposed the total psychomotor response time into an evaluative and a motor component (Edwards et al., 2007). The study found an inhibition of stimulus processing during the middle of the cardiac cycle, while the motor response elicitation remained unaffected. Because the effect was highly dependent on the modality of the go-stimulus, this finding requires further evaluation.

Thus, previously conducted studies show that there is evidence for modulatory effects of baroreceptors on CNS functions, but their distinct functions and pathways remain unclear. However, effects on simple cognitive functions, such as psychomotor responses, may not serve as a valid indicator for baro-afferent signal transmission for at least two reasons: Firstly, these functions rely on conscious processing and active response execution. Therefore, the participants' performance is highly related to their motivational and emotional states. Secondly, it is not clear, which components of psychomotor responses are actually affected by baro-afferent neural feedback. In conclusion, it is reasonable to investigate a cognitive variable that is influenced by baroreceptor loading, but is independent of participants' active cooperation.

1.3.3 Baroreceptor Impact on Electrocortical Functions

Beyond these behavioral measures, there are also modulatory effects of baro-afferent neural feedback on psychophysiological variables. These findings generally support the hypothesis of a baroreceptor-induced CNS inhibition. For instance, in the field of electrocortical potentials, the Contingent Negative Variation (CNV) that can be observed in anticipation of a go-stimulus represents a slow potential that is assumed to reflect cortical excitability (Birbaumer, Elbert, Canavan & Rockstroh, 1990). An increase of arterial baroreceptor loading by either pharmacological or mechanical manipulation resulted in a reduction of the CNV amplitude, suggesting a lower cortical excitability during baroreceptor stimulation (Elbert, Roberts, Lutzenberger & Birbaumer, 1992; Larbig, Elbert, Rockstroh, Lutzenberger & Birbaumer, 1985; Rau, Pauli, Brody, Elbert & Birbaumer, 1993), for review see: Elbert and Rau (1995). These results may be interpreted as a cortical inhibition effect of arterial baroreceptor loading. Nevertheless, the appearance of CNV modulation requires a specific task and a controlled experimental setting and cannot be observed in a resting condition without cognitive demand, which may counteract with baroreflex processes.

One electroencephalographic component that reflects cardiac activity and does not require a specific foreground task is the heartbeat-evoked potential (HEP) (Schandry, Sparrer & Weitkunat, 1986). This event-related potential can be observed at a latency of 250-550 ms after an actual R-wave (Montoya, Schandry & Muller, 1993). The HEP is associated with an individual's ability of heartbeat perception (Pollatos & Schandry, 2004) and the attentional

focus on intero- vs. exteroceptive signals (Montoya et al., 1993). Furthermore, the HEP has been found to originate from the Right insula, the Prefrontal cortex and the left Secondary somatosensory cortex (Pollatos et al., 2005). These structures are also active while participants are performing heartbeat perception tasks (Critchley et al., 2004) and pharmacologic baroreceptor stimulation (Henderson et al., 2004). As summarized by Pollatos and colleagues (2005), baro-afferent neural traffic may first reach the NTS and then be relayed over the Parabrachial nucleus and the Thalamus to the Insula, one of the cortical centers involved in body-related information processing. Thus, the HEP may serve as a background methodology to assess ascending neural traffic originating from the cardiovascular system. However, it is unlikely that the HEP only reflects baro-afferent traffic, since it is a product of cortical projections rather than of brainstem-relayed neural transmission. Therefore, it is not possible to differentiate between pre-attentive baro-afferent feedback and attentive processed signals via the HEP technique.

1.3.4 Pain Perception and Startle Responsiveness

A major adaptive function of arterial baroreceptor impact on higher CNS circuits is the modulation of pain perception. In a stressful and critically dangerous situation, an individual's autonomic stress response leads to a raise of blood pressure. This rise in blood pressure leads to an increase of arterial baroreceptor loading and therefore to a reduction of pain perception (France, 1999). The first study on the effects of blood pressure elevations on pain perception investigated rats' responses to noxious stimuli (Dworkin, Filewich, Miller, Craigmyle & Pickering, 1979). The authors found that a blood pressure elevating medication increased rats' pain threshold. However, these effects were not observed for baro-denerved individuals.

There is accumulating evidence for similar relationships between blood pressure and pain perception in humans. Lower pain sensitivity was found in chronically hypertensive patients (Bruehl, Carlson & McCubbin, 1992; Sheps et al., 1992). The importance of the inhibitory effect of baroreceptor loading was emphasized by studies that found the same relationship in healthy participants across the cardiac cycle. Nociception flexion responses to painful stimuli were lower in the early cardiac cycle phase, during which the arterial pulse wave increases the baro-afferent neural traffic (Edwards et al., 2003; Edwards, McIntyre, Carroll, Ring & Martin, 2002; Edwards, Ring, McIntyre & Carroll, 2001). These studies demonstrated that central

inhibitory effects that occur very rapidly can be investigated by measuring the modulation of reflex responsiveness via EMG.

As summarized by Nyklicek and colleagues (2005), the inhibition effect of baroreceptor stimulation does not only affect pain perception, but may generally attenuate the responses to potential stressors, such as the responses to acoustic startle stimuli. Accordingly, a static stimulation of arterial baroreceptors via a neck cuff was found to reduce startle eye blink responses in human participants (Elbert & Rau, 1995; Rau, Lutzenberger & Elbert, 1989). The authors hypothesized that ascending baroreceptor feedback to mesencephalic reticular structures may play a role in the modulation of startle responses, because these structures have previously been shown to be involved in the inhibition of the startle response (Leitner, Powers & Hoffman, 1980; Leitner, Powers, Stitt & Hoffman, 1981). Furthermore, the amplification of baro-afferent neural traffic across the cardiac cycle by the PRES-(Phase-related external suction) neck cuff technique (Rau et al., 1994; Rau et al., 1992) also resulted in lower startle-responsiveness during maximal arterial baroreceptor stimulation in the early cardiac cycle phase (Nyklicek et al., 2005). The startle methodology seems to be an adequate measure for baro-afferent neural traffic, because it has some methodological advantages compared to other methods that have been used to measure baro-afferent neural traffic: (i) the startle response occurs with a very short latency after the stimulus and can be observed independently from the individual's conscious cooperation (Koch, 1999), (ii) the startle responsiveness is modulated by several psychophysiological processes (Filion, Dawson & Schell, 1998), and (iii) the startle response magnitude can be quantified easily and has a good signal-to-noise ratio (Blumenthal et al., 2005).

1.3.5 Conclusion

Earlier studies suggest that an experimentally induced increase of arterial baroreceptor loading via neck cuff techniques may attenuate startle eye blink responsiveness. The current knowledge is based on laboratory findings that involve mechanical stimulation of arterial baroreceptors. However, these methods represent an unecological context for baro-afferent neural traffic. For instance, it cannot be excluded that acoustic or somatosensory feedback induced by the neck cuff device may interact with startle responsiveness. For example, acoustic or somatosensory feedback may act as prepulses, and prepulses have been shown to

attenuate the startle response magnitude (Blumenthal, 1999). To address this limitation of previous studies on baro-afferent neural traffic, I have developed a method, which employs acoustic startle stimulations across the cardiac cycle (early vs. late cardiac cycle phase) under resting conditions. In this paradigm, only naturally occurring effects within the cardiac cycle, such as loading and unloading of arterial baroreceptors by the arterial pulse wave, may have an impact on startle eye blink responsiveness. The following last paragraph of the General Rationale will give an overview about the aims, empirical evidence and interpretations of the Original Research Articles.

1.4 Cardiac Modulated Startle: Empirical Evicence

1.4.1 Cardiac Modulation of Startle Eye Blink and its Disappearance in Autonomic Neuropathy

Study 1 (38 healthy volunteers) provides evidence that the startle response magnitude to acoustic startle stimuli is attenuated when they are presented during the early cardiac cycle phase (R-wave +230 ms), compared to a presentation of startle stimuli during the late cardiac cycle phase (R +530 ms). This cardiac modulation effect on startle (CMS) may result from the central inhibition of increased baro-afferent neural traffic during the cardiac systole, caused by the arterial pulse wave. To investigate whether arterial baro-afferent feedback is responsible for the startle inhibition during the early cardiac phase, *Study 2* investigated the same effect in diabetic patients with normal vs. diminished baroreflex-sensitivity (BRS). A long duration of diabetes disease often results in a cardiac autonomic neuropathy, the earliest sign of which is a reduction of BRS (Frattola et al., 1997; Gulli, Fattor & Marchesi, 2005; Weston et al., 1998; Weston et al., 1996). A group of 31 diabetics was divided into a group of 13 patients with a BRS lower than 6 ms/mmHg and a group of 18 patients with a BRS higher than 6 ms/mmHg, according to a commonly used cut-off value (La Rovere, Bigger, Marcus, Mortara & Schwartz, 1998). Startle stimuli were again presented to both groups during the early (R-wave +100-400 ms) and the late cardiac cycle phase (R +401-800 ms). Diabetic patients with a normal BRS (> 6 ms/mmHg) showed the same startle response pattern as healthy volunteers. However, the diabetic group with diminished BRS (< 6 ms/mmHg) did not show a startle attenuation during the early cardiac phase. It can thus be concluded that intact baro-afferent neural feedback is required to find the cardiac modulation effect on startle eye blink. It is justified to assume an association between the integrity of the baro-afferent

pathways and the described modulatory effect of startle responsiveness. However, two important questions remained unclear: First, it is likely that the arterial pulse wave and the arterial baroreceptors stimulated thereby are responsible for the modulation, but the modulation could also reflect a simple effect of prepulse inhibition (PPI) of startle eye blink, because even cardiac activity may act as a prepulse. Second, the cardiac modulation effect may be relayed via distinct neural connections between the responsible pathways, but it could represent a general attenuation of sensory input, as well. The following chapters address these questions.

The here described method for investigating baro-afferent feedback is innovative, because the observed effect fully relies on brainstem-relayed reflexes and thus appears pre-attentively. Both reflex pathways that are involved are well described. The startle reflex consists of three synapses: at the Cochlear root neurons, the Nucleus reticularis pontis caudalis (NRPC), and the Facial motor nucleus (Davis, Walker & Lee, 1999). The baroreflex is also relayed via three synapses: from the arterial baroreceptors to the Nucleus tractus solitarius (NTS), the Nucleus ambiguus (NA), and the Cardiac pacemaker (Jänig, 2006). However, up to now a direct connection between the two reflex pathways is not known. A more detailed knowledge about the responsible neural pathways is required to understand the CMS effect and the underlying processes of body and brain signal transmission.

1.4.2 Lateralization Effects

One neuroscientific similarity between the processing of emotional-relevant and interoceptive information is their right-hemispheric advantage. Early studies have shown that affective stimuli are processed more accurately when they are presented to the left ear, which projects mainly onto right-hemispheric brain structures (Carmon & Nachshon, 1973; Haggard & Parkinson, 1971; Joseph, 1988). Accordingly, the affective startle modulation paradigm, in which the affective valence of a foreground stimulus modulates the startle responsiveness, shows a specificity for lateral stimulation to the left ear (Bradley, Cuthbert & Lang, 1991; Bradley, Cuthbert & Lang, 1996). Furthermore, the information processing of interoceptive signals is improved when right-hemispheric functions are activated (Katkin & Reed, 1988; Weisz, Balazs & Adam, 1994). Functional imaging studies support these behavioral data. These studies found a right-hemispheric activation of the Anterior insula and the Anterior

cingulum when participants performed interoceptive paradigms (Critchley et al., 2004; Pollatos et al., 2007a), and during pharmacologic baroreceptor stimulation (Henderson et al., 2004). *Study 3* discovered lateral specificities within the cardiac modulation of startle eye blink, similar to those that were found in the affective startle modulation paradigm before. In this study, startle stimuli were not only presented at a latency of 230 and 530 ms relative to the R-wave, but also at R +0 ms and R +100 ms to discover early effects of the cardiac cycle on startle responsiveness. Furthermore, startle stimuli appeared lateralized both at the left and at the right ear, as previously applied in a pilot study with 22 participants (Schulz, 2007). Here, 37 healthy volunteers were investigated.

The results confirmed the presence of the CMS effect we described in *Study 1* and *Study 2*. However, this effect was only visible at a latency of R +230 ms, compared to all other conditions. These results are in line with earlier results that showed maximal baro-afferent attenuation effects at this latency within the cardiac cycle (Edwards et al., 2001; Edwards, Ring, McIntyre, Winer & Martin, 2009). Additionally, the effect could only be observed in left-ear stimulation. That suggests a right-hemispheric advantage in processing modulation-relevant neural traffic because of the lateral crossing of the primary acoustic startle circuit at the level of the modulation-sensitive NRPC structure (Davis et al., 1999). Based on the finding that right-hemispheric structures are more involved in relaying neural signal transmission originating from the cardiovascular system, the same may also modulate the startle responsiveness at the ipsilateral NRPC. Whether the startle reflex is modulated via direct or indirect projections cannot be answered by this study. Neither can the question be answered, which structures are actually involved in cardiac startle modulation. However, the Amygdalae play a role in the affective startle modulation (Angrilli et al., 1996; Funayama, Grillon, Davis & Phelps, 2001) and in relaying interoceptive neural transmission (Cechetto & Calaresu, 1984; Cechetto & Calaresu, 1985). Therefore, I suggest that they may also contribute to the cardiac modulation effect on startle. Of course, this question requires further exploration, but the results of *Study 3* have shown that a specific neural pathway may be responsible for the CMS effect rather than a general sensory inhibition by baro-afferent neural traffic. The specificity of the CMS effect to left-ear stimulation also argues against the responsibility of PPI for the observed cardiac modulation. Furthermore, because of the here described lateralization effect, it is likely that the structures mediating the CMS effect are

located on a higher level than the brainstem (e.g., Limbic system or higher), suggesting a top-down modulation.

1.4.3 Higher Cognitive Processing

Studies 1, 2 and *3* provide evidence that startle responsiveness is modulated across the cardiac cycle and that baro-afferent neural traffic may be responsible for that effect. Furthermore, I suggested that higher CNS structures may also be involved in the cardiac modulation of startle responsiveness, although the empirical data so far does not allow for specific conclusions. However, I postulate that the CMS paradigm is an alternative to classical interoceptive paradigms that rely on participants' active cooperation. While the integrity of baro-afferent signal transmission is clearly reflected by the CMS paradigm, it is yet unclear whether the pre-attentive modulation of startle is also accompanied by an altered attentive processing and perception of the startle stimuli. Most psychological domains that operationalize interoception assume subjective awareness of bodily states. Therefore, to ensure comparability between the CMS method and other interoceptive paradigms, *Study 4* tested whether cardiac modulation of startle is also reflected by a modulated cognitive processing of startle stimuli. 25 healthy volunteers underwent a modified study protocol, in which startle stimuli were presented at 230 and 530 ms after the R-wave. Here, the startle stimuli were presented in three different intensities (85, 95 and 105 dB(A)) to provide a conclusive reason for the participants to discriminate stimuli. Earlier studies showed that higher startle intensities result not only in an increased startle eye blink responsiveness, but also in faster psychomotor responses to those stimuli (Carlsen, Dakin, Chua & Franks, 2007). Additionally, in other startle modulation paradigms, startle eye blink response magnitude correlated highly with the subjectively perceived intensity of the presented startle probes (Swerdlow, Blumenthal, Sutherland, Weber & Talledo, 2007; Swerdlow, Geyer, Blumenthal & Hartman, 1999; Swerdlow et al., 2005). Thus, in *Study 4* participants were asked to continuously press a home button until they heard a startle noise and then to respond as fast as possible by pressing the response button. With this technique it is possible to discriminate between the evaluation and the motor response component of the psychomotor response time. Afterwards, participants evaluated the subjective intensity of the stimuli via an Electronic Visual Analog Scale (EVAS).

Replicating earlier findings, *Study 4* found a facilitating effect of higher stimulus intensity and the late cardiac cycle phase on startle eye blink. Furthermore, we found that the cardiac cycle influenced behavioral measures as well. Participants judged those stimuli that were presented during the early cardiac cycle phase as less intense. We did not find a cardiac cycle effect on the total psychomotor response time, although this was repeatedly found for non-startling stimuli (Birren et al., 1963; Edwards et al., 2007; Stewart et al., 2006). However, the total psychomotor response time may be decomposed into the evaluation component (time from stimulus onset to initiation of a motor response) and the motor component (time from initiation to the completion of a motor response) (Doucet & Stelmack, 1999; Doucet & Stelmack, 2001; Jensen, 1979). A separate analysis for the evaluation (= reaction time: RT) and the motor component (= movement time: MT) revealed converse effects of the cardiac cycle on both components. The inhibition of the RT component during the early cardiac cycle phase is in line with earlier results that showed an impairment of the RT and the total response time, but no effect of the cardiac cycle on the MT (Edwards et al., 2007). In contrast to this, in *Study 4* an acceleration of the MT during the early cardiac cycle phase was found. One explanation for this effect might be an adaptive mechanism under stressful conditions, during which increased baro-afferent traffic is expected: the organism may then benefit more from the execution of motor evasive responses (fight-or-flight), but less so from the detailed cognitive evaluation of incoming environmental stimuli.

Overall, *Study 4* revealed multiple effects of the cardiac modulated startle on behavioral and therefore, on higher cognitive functions. The CMS effect is associated with an altered attentive processing and perception of startle stimuli that are elicited in different cardiac cycle phases. Furthermore, the converse effects of the cardiac cycle on RT and MT provide more evidence for a specific neural pathway of baro-afferent neural feedback. Although simple psychomotor responses and subjective intensity judgments clearly require attentive resources and thus higher cognitive processing, it is difficult to link the observed effects to distinct brain regions (Swerdlow et al., 2007; Swerdlow et al., 2005). Nevertheless, the influence of cardiac modulated startle on higher cognitive functions increases the comparability of the CMS paradigm, which mainly depends on brainstem reflexes, to other common interoceptive paradigms that require conscious awareness of bodily processes. One conclusion of this study is the differentiation of the CMS effects into (i) a brainstem-relayed modulation of the startle eye blink, and (ii) a cortical-processed modulation of subjective intensity and psychomotor

response time. This differentiation is an important advantage of the CMS paradigm compared with the HEP (Schandry et al., 1986), which only reflects cortical processing of heartbeat-related signal transmission. It is likely that under some circumstances, the baro-afferent feedback is intact, but a disruption in the cortical processing of bodily signals may occur. In such circumstances, which will also be considered in Paragraph 1.5, neural afferent traffic could only be measured via the CMS technique.

1.4.4 Cardiopulmonary Baroreceptors

A wide range of inhibitory effects of arterial baroreceptor stimulation on reflex responsiveness and higher cognitive functions are described in literature (Birren et al., 1963; Edwards et al., 2001; Nyklicek et al., 2005). The results of *Studies 1, 2, 3* and *4* contribute to this evidence. However, the arterial baroreceptor is not the only receptor type that mediates sensory information originating from the cardiovascular system to CNS structures. As described above, beside the quick-responding 'high-pressure' system, which mainly relies on the baroreceptors located in the aortic branch and the carotis, there is also a 'low-pressure' system with its cardiopulmonary baroreceptors located in the venous system and the cardiac artria. It is likely that both systems work closely together in cardio-afferent signal transmission for at least two reasons: Firstly, the responsible and involved neural pathways for both baroreceptor systems are very similar for brainstem (Aicher, Kurucz, Reis & Milner, 1995; Jänig, 2006; Jeske, Reis & Milner, 1995) and cortical neural networks (Henderson et al., 2004; Kimmerly et al., 2005). Secondly, studies investigating the 'low pressure' system found inhibiting effects of cardiopulmonary baroreceptor loading that were comparable to the inhibiting effects of the arterial baroreceptors (Vaitl & Gruppe, 1990). However, there is no research on interactions of both baroreceptor-types: both systems may contribute to the same effect or interact in a more complex way. We addressed this question in *Study 5*. The original protocol of *Study 1* was applied to 12 healthy male volunteers, with acoustic startle stimuli being presented at latencies of 230 and 530 ms after the R-wave to represent two different loading conditions of arterial baroreceptors. Furthermore, the loading status of the cardiopulmonary baroreceptors was manipulated by a Lower Body Negative Pressure (LBNP) intervention. The LBNP procedure induces a variation of central vascular blood volume without a substantial influence on the systolic blood pressure (SBP) (Kitano, Shoemaker, Ichinose, Wada & Nishiyasu, 2005; Laszlo, Rossler & Hinghofer-Szalkay, 1998; van Hoeyweghen et al., 2001). Using LBNP, an unloading of cardiopulmonary baroreceptors can

be achieved by a higher level of LBNP suction. Here, the participants were each exposed to four different suction conditions of 0, -10, -20 and -30 mmHg in a counterbalanced order twice. In addition to EMG startle responsiveness to the acoustic stimuli, we measured psychomotor responses to each noise. Due to technical issues, the extensive apparatus we used to collect psychomotor response times in *Study 4* could not be used in the LBNP chamber. Thus, it was replaced by a simple handheld button that participants were asked to press in response to the startle stimulus. However, with this technique it is not possible to decompose the total response time into reaction time (RT) and movement time (MT).

Study 5 again showed an attenuation of startle eye blink responsiveness when startle stimuli were presented during the early cardiac cycle phase (R +230 ms) compared to the late cardiac cycle phase (R +530 ms), presumably caused by increased arterial baroreceptor loading. Furthermore, an unloading of cardiopulmonary baroreceptors via the four LBNP conditions resulted in an enhancement of startle eye blink responsiveness. A contrast analysis revealed a linear trend over all four conditions with increased startle eye blink magnitudes from zero to maximal LBNP level, indicating a dose-response relationship between the unloading of cardiopulmonary baroreceptors and startle responsiveness. The LBNP condition of -30 mmHg is comparable to a blood loss of approximately 500 ml. In this stressful and potentially dangerous context, an enhancement of protective reflexes may be adaptive for the organism (Koch, 1999). However, we did not observe an interaction of arterial and cardiopulmonary baroreceptor loading conditions. Therefore, it can be concluded that the central inhibition of both baroreceptor types have summative effects on eye blink responsiveness. However, this result has to be evaluated carefully, since earlier studies repeatedly found interactions of both systems (Brown, Hecht, Neundorfer & Hilz, 2003; Cooper & Hainsworth, 2001; Victor & Mark, 1985). One possible point of criticism of our study is that the experimental manipulations for inducing the loading and unloading of arterial and cardiopulmonary baroreceptors were not fully comparable. The effects we found across the cardiac cycle represent a phasic stimulation of arterial baroreceptors, but different LBNP levels represent a static stimulation of cardiopulmonary baroreceptors. Additionally, neither the cardiac cycle, nor the different LBNP levels had an effect on the psychomotor response times. This result is not surprising, since we did not observe an effect of the cardiac cycle on the total response time in *Study 4* either and *Study 4* comprised a larger study sample and therefore a higher power than *Study 5*. A potential decomposing into RT and MT might have resulted in an

effect of either baroreceptor loading condition. However, a decomposing was not possible due to technical restrictions of the LBNP device. Furthermore, modulatory effects of the cardiac cycle on either RT or MT were only found in lower startle stimulus intensities than the 105 dB(A) used here. Nevertheless, our participants reported an increase in subjective arousal during higher LBNP suction. This also implies an impact of cardiopulmonary baroreceptor loading on cognitive processes. In summary, *Study 5* showed that cardiopulmonary baroreceptor loading has inhibiting effects on eye blink responses comparable to those of arterial baroreceptor loading, but had no effect on psychomotor response time.

Overall, the startle paradigm is feasible for reflecting neural traffic originating from cardiopulmonary and arterial baroreceptors. In *Study 5*, no interaction between the loading conditions of arterial and cardiopulmonary baroreceptors was found. It can thus be concluded that startle eye blink responsiveness is modulated by multiple sources of afferent neural feedback from the cardiovascular system. This observation has some methodological implications: Firstly, further studies should investigate whether other sources of visceral afferent neural traffic may have an impact on startle responsiveness, as well, or may interact with the here described effects. Secondly, under standardized experimental conditions, it has to be ensured that groups of participants systematically only differ in one source of afferent neural traffic and that other potential sources of variance are varied orthogonally. However, the modulation of startle responsiveness across the cardiac cycle indicates a rapid-occurring pre-attentive modulation effect of afferent arterial baroreceptor feedback. This pre-attentive modulation is also associated with attentive impacts of cardiac modulated startle. Our manipulation of cardiopulmonary baroreceptors also influenced startle eye blink responses and higher cognitive functions. However, in the static LBNP intervention, it is impossible to separate rapid pre-attentive from attentive modulation effects. We cannot exclude that the startle modulation effect by LBNP suction is not a result of cardiopulmonary baroreceptor stimulation, but an effect of the arousal level that was induced by LBNP. Future studies are needed to resolve this question. However, the characteristics of the CMS effects of either arterial and cardiopulmonary baroreceptor loading were comparable and similar neural pathways have been identified for both systems (Jänig, 2006). Therefore, it is likely that findings regarding the effect of the cardiac cycle on startle, which is hypothesized to reflect different conditions of arterial baroreceptor afferent feedback, may be partially generalized to the cardiopulmonary baroreceptor system. In conclusion, cardio-afferent neural feedback of

various sensoric sources of the cardiovascular system can be measured via the CMS paradigm and this cardio-afferent neural feedback represents the neural basis for cardiac interoception.

1.5 Further Implications

1.5.1 Neural Circuits

As summarized above, the two important neural circuits involved in the CMS effect are (i) the primary acoustic startle circuit and (ii) the arterial baroreflex circuit. Neural information within the primary acoustic startle circuit is transmitted via the cochlear root neurons, the Nucleus reticularis pontis caudalis (NRPC), and the Facial motor nucleus (Davis et al., 1999). The parasympathetic branch of the baroreflex circuit involves synapses in the Nucleus tractus solitarius (NTS), the Nucleus ambiguus (NA), and the Cardiac pacemaker (Jänig, 2006). Regarding the primary acoustic startle circuit, it is assumed that all modulatory functions affect the startle response on the level of the NRPC. For instance, in the affective startle modulation paradigm, in which the valence of a foreground stimulus is associated with the startle response magnitude, the NRPC receives projections from the Central amygdala, the Bed nucleus of the stria terminalis (Davis, 2006), and the Periaqueductal gray (Koch, 1999). In the prepulse inhibition of startle, higher structures impact on the NRPC via the Pedunculopontine tegmental nucleus. The NTS has been suggested to be one of the most important structures in visceral- and baro-afferent signal transmission, because it relays bodily signals of various origin (Jänig, 2006). However, for two reasons it is difficult to speculate about distinct neural connections between the startle and the baroreflex circuit: First, the NRPC receives afferents of many different structures located in the brainstem, Basal ganglia and Limbic system (Davis, 2006; Koch, 1999). Second, the NTS projects onto many different brainstem-located and higher structures, such as the Caudal and Rostral ventrolateral medullae (Jänig, 2006), the Lateral parabrachial nucleus (Beckstead, Morse & Norgren, 1980; Loewy & Burton, 1978), the Limbic System (Ricardo & Koh, 1978), and (via the Lateral ventroposterior thalamus) onto the Insular cortex (Cechetto & Saper, 1987), as summarized by Dembowski and Seller (1995). The fact that the NTS also receives afferents of most of the structures, onto which it projects, makes it even more complicated to identify structures responsible for a possible connection.

A direct link between the primary synapses of both circuits has not been described in literature yet. Any possible mediating pathway somehow involves structures of the Limbic system. As stated above, the Central amygdala nucleus and the Bed nucleus of the stria terminalis substantially contribute to the affective startle modulation on the level of the NRPC (Davis, 2006; Davis et al., 1999). Furthermore, one major projection of the NTS reaches the Lateral parabrachial nucleus, which is ascendingly connected with the Central amygdala nucleus and the Bed nucleus of the stria terminalis (Fulwiler & Saper, 1984). This could be a first hint for a neural model integrating the startle and the baroreflex circuit. The importance of these two structures is emphasized even more by the fact that both are also connected with the NA, the Dorsal motor nucleus of the vagus, and the Locus coeruleus (LC) (Davis, 2006), which all play a direct or indirect role in mediating baro-afferent neural traffic. Nevertheless, it remains unclear whether a rapid-occurring effect like the CMS may be relayed over a neural pathway that involves limbic structures.

1.5.2 Interoception in Psychosomatic Disorders

The aim of this work is to establish a new methodology to assess body-related afferent signal transmission. Perception of bodily signals plays a crucial role in the etiology of multiple psychiatric and psychosomatic disorders, especially in panic and somatoform disorders. Accordingly, there is a long history of research addressing the altered ability of clinical samples in interoception, which is usually assessed via heartbeat-counting (Schandry, 1981b) or heartbeat-discrimination paradigms (Whitehead et al., 1977). Earlier studies investigating the interoceptive ability of patients suffering from panic disorders either found no difference between those patients and healthy controls or an improved interoceptive ability in panic disorder patients (Ehlers & Breuer, 1992; Ehlers & Breuer, 1996; Ehlers et al., 1995). Moreover, the comparison of interoceptive accuracy in patients suffering from somatoform disorders and healthy controls did not reveal consistent results. They range from increased (Dahme, Dedic & Ungruh, 1997), over zero results (Barsky et al., 1995), to impaired interoceptive accuracy (Mussgay et al., 1999).

Thus, there is no consistent conclusion about the relationship between interoceptive accuracy and body-related disorders. Early works assumed that psychosomatic patients have a superior body perception ability. (Tyrer, 1973). Others emphasized the role of attentional biases in

patients with these disorders towards bodily signals without having a naturally increased interoceptive accuracy (Ehlers et al., 1995). This would also explain the dissociation between patients' subjective and objective ability of body perception (Khalsa et al., 2008). The theory of somatosensory amplification, an influential cognitive model for increased awareness to bodily processes, also emphasizes the role of fear of body sensations in somatoform and panic disorders (Barsky, 1992; Barsky, Goodson, Lane & Cleary, 1988; Barsky & Wyshak, 1990). This model postulates a positive feedback cascade that begins with a minor sensation of a bodily process. This sensation causes fear and worries about the actual health status and leads to an increased awareness of bodily signals, which in turn increases the likelihood of the perception of new body sensations. Thus, it can be concluded that cognitive factors, such as attentional biases and fear, may have a strong influence on body perception. Given that experimental factors, such as the strictness of instruction, motivation and effort, substantially influence the interoception accuracy scores (Ehlers et al., 1995), past interoceptive research has not been able to adequately reveal the role of body perception accuracy in the genesis of panic or somatoform symptoms. Furthermore, recent studies neglect the role of the neural correlates of body perception and therefore body-related afferent signal transmission.

The above mentioned cognitive factors play an essential role in the aetiology of panic and somatoform disorders, which both have substantially overlapping symptoms (Barsky, Cleary, Sarnie & Ruskin, 1994). Therefore, it is unlikely that the altered body perception in these patients can be explained with a biological model that simply relies on the visceral-afferent signal transmission (Cioffi, 1991). However, it was recently found that patients suffering from somatoform disorders have a pathological baroreflex integrity (Laederach-Hofmann, Ruddel & Mussgay, 2008), which may suggest a failure in baro-afferent signal transmission. However, a diminished BRS cannot reveal whether the afferent or the efferent branch of the baroreflex may be dysfunctional in these patients. A feasible approach for investigating possible dysfunctions in baro-afferent neural feedback would be a comparison of response patterns in the CMS paradigm of somatoform patients and healthy individuals. This paradigm should consist of a startle presentation during the early and the late cardiac cycle phase and the dependent variables (i) startle eye blink, (ii) subjective intensities and/or (iii) psychomotor response times. For instance, the cardiac modulation of startle eye blink could be identical in psychosomatic patients and healthy participants, but the modulation of subjective intensity or psychomotor responses could be different or absent. In this case, it could be concluded that

the baro-afferent neural traffic itself is not disrupted, but there may be a failure in visceral-afferent neural transmission to higher cognitive centers. Therefore, the CMS methodology may not only have the potential to test for an integrity of baro-afferent signal transmission, but may also allow for conclusions about the level of disruption.

1.5.3 Baro-Afferent Neural Transmission under Stress

Stress is considered to be an eliciting and maintaining factor for multiple psychosomatic disorders (McEwen, 2004). Although the functional role of body perception in the genesis of psychosomatic symptoms remains unclear, stress may serve as a link between both. In animal studies it has been shown that early life (Caldji et al., 1998; Liu, Caldji, Sharma, Plotsky & Meaney, 2000) or chronic stress (Flugge, van Kampen, Meyer & Fuchs, 2003) may cause a reduction of the expression of α_2-adrenoreceptors in the LC and the NTS. The major function of these α_2-adrenoreceptors is the inhibition of norepinephrine release from the LC (Hein, 2001). Activation of α_2-adrenoreceptors in the Ventrolateral medulla reduces sympathetic activity, while the same activation in the Dorsovagal nucleus enhances parasympathetic activity (Unnerstall, Kopajtic & Kuhar, 1984). Thus, α_2-adrenoreceptors are an important source of the homeostatic downregulation of the autonomic nervous system (ANS). A dysfunctionality of α_2-adrenoreceptors leads to a chronic hyperactivation of the ANS (Flugge, Kramer & Fuchs, 2001). An individual history of stress may therefore contribute to a disruption of baro-afferent signal transmission, because one brain structure that might be affected by a stress-induced reduction of α_2-adrenoreceptor density is the NTS, which represents the most important brainstem center for visceral-sensory input. In line with this, research has shown that acutely induced hyperactivity of the ANS by administration of the α_2-antagonist Yohimbine in rats affects the metabolism in the NTS, which led to an abnormal alimentary behavior (Myers, Banihashemi & Rinaman, 2005). A modification of alimentary behavior may serve as an indicator for altered visceral-afferent signal transmission in animals. This may imply that a dysregulation of the α_2-adrenoreceptor system has the potential to disrupt visceral-sensory signal transmission, possibly at the level of the NTS, which then also results in a behavioral modification. Unfortunately, there is no comparable data available from human studies. Nevertheless, one study showed that subjectively reported chronic stress is positively correlated with an activation of brain structures that are associated with visceral-sensory processing, such as the Insular cortex (Rosenberger et al., 2009). This result underlines the importance of chronic stress in the development of visceral malsensations.

The CMS paradigm may be feasible to reflect the integrity of baro-afferent neural traffic in the case of impaired α_2-adrenoreceptor density or activity. For instance, it is reasonable that the CMS response patterns may differ in individuals with varying α_2-adrenoreceptor density in the NTS. However, it is difficult to measure this receptor density in vivo. Furthermore, a pharmacologic induction of an α_2-adrenoreceptor hypoactivity, which leads to an acute ANS hyperactivity (e.g., Philippsen et al., 2007), may also be reflected by an altered CMS pattern compared with a resting condition. An α_2-adrenoreceptor hypoactivity can be induced with an α_2-adrenoreceptor antagonist, such as Yohimbine. It is known that Yohimbine has an anxiogenic effect that potentiates the startle response (Morgan et al., 1993). Thus, another open question is whether a possible modulation pattern of the CMS effect is independent of the total startle magnitude. Actually, results of a pilot study demonstrated a general increase in startle response magnitude during Yohimbine administration compared to a placebo condition, while the CMS effect could be only observed during placebo, but not during Yohimbine administration (Schulz, Philippsen & Schächinger, 2007). This finding implicates an involvement of α_2-adrenoreceptors in the CMS effect, presumably at the level of the NTS, but it requires further exploration due to a small sample size (n=8). Overall, the investigation of chronically and acutely stressed individuals, who suffer from an α_2-adrenoreceptor hypoactivity, may contribute to the understanding of the association of stress and the processing of body-related signals. Clinical studies should address this question in the future.

Chapter II:

Cardiac Modulation of Startle Eye Blink

(Schulz et al., 2009a)

Co-Authors: Johanna Lass-Hennemann, Frauke Nees, Terry D. Blumenthal, Willi Berger & Hartmut Schächinger.

2.0 Abstract

As an alternative to interoceptive paradigms that depend on the participants' active cooperation, two studies are presented to show that startle methodology may be employed to study visceral afferent processing. The first study of 38 volunteers showed that startle responses were smaller when elicited during cardiac systole as compared to diastole. In the second study, 31 diabetic patients were divided into two groups, having normal or diminished (< 6 ms/mmHg) baroreflex sensitivity (BRS). Patients with normal BRS showed the same results found in healthy volunteers. Diabetic patients with diminished BRS did not show this pattern. Because diminished BRS is an indicator of impaired baro-afferent signal transmission, it is concluded that cardiac modulation of startle is associated with intact baro-afferent feedback. Thus, pre-attentive startle methodology is feasible to study visceral afferent processing originating from the cardiovascular system.

***Keywords*:** Autonomic Neuropathy, Baroreflex, Diabetes, Interoception, Startle Eye Blink, Visceral Perception

2.1 Introduction

Visceral afferent signal transmission and processing play a crucial role in emotion (Berntson, Sarter & Cacioppo, 2003; Critchley et al., 2004). Visceral sensations have been linked to brain areas that modulate emotional processes, such as the Anterior insula and Anterior cingulate, and visceral sensations are integrated in the subjective experience of emotion (Critchley et al., 2004; Wiens, 2005). Furthermore, visceral afferent signals are assumed to

play a role in consciousness (e.g., Damasio's somatic marker hypothesis links internal body information to self consciousness: Damasio, 2003), and symptom genesis (e.g., Eley, Stirling, Ehlers, Gregory & Clark, 2004). Enhanced interoception has been found in anxiety or somatoform disorders (Ehlers & Breuer, 1996).

A participant's willingness to cooperate in a visceral afferent signal transmission task may influence task performance. Furthermore, task engagement (e.g., counting) may limit attentional resources directed towards visceral sensations. However, many tasks currently used to study human visceral afferent processing are characterized by such limitations. The present study proposes the use of pre-attentive startle methodology as a supplementary technique in studying the processing of visceral afferents.

Visceral sensory processes may be induced by mechanical or electrical stimulation of visceral structures, such as inflation of balloons to stimulate the stomach (Dworkin, 2000), or by electrical stimulation of the gastrointestinal system via endoscopes (Drewes et al., 2006). Arterial baroreceptors may be stimulated mechanically by neck cuffs (Rau & Elbert, 2001). However, such procedures may direct attention to external devices (endoscope, neck cuff) or reduce comfort, and can be expensive, as well as being rather sensitive to stimulator and sensor displacement.

Other tests are based on the ability to intentionally focus on spontaneous cardiac activity, such as the heartbeat tracking task (Schandry, 1981a), or intero-exteroceptive discrimination tasks (e.g., Brener & Kluvitse, 1988; Whitehead et al., 1977), which reflect the ability to intentionally perceive and report signals related to cardiac activity. Such 'foreground' tasks obviously require a subject's awareness and cooperation, thereby they occupy and direct attention towards cardiac phenomena. Alternative test strategies may be based on heartbeat evoked scalp potentials (HEP). Electro-cortical activity locked to spontaneous cardiac activity is visible as a positive waveform in a relatively large interval, ranging from 300 to 600 ms after the R-wave (Schandry & Montoya, 1996). The advantages of this technique are that it may be recorded in the 'background,' it does not require active cooperation, and it involves processes above the brainstem level. This is supported by the finding that the HEP is related

to performance scores in heartbeat awareness tasks (Pollatos & Schandry, 2004). Furthermore, the HEP has been linked to integrity of visceral-afferent pathways of the autonomic nervous system (Leopold & Schandry, 2001). Although clearly related to periodic cardiac activity, it is difficult to link the HEP to distinct cardiac or vascular hemodynamics, such as systole or diastole, because of the HEP's relative broad temporal distribution over the cardiac cycle (e.g., a HEP of 300-600 ms will partly overlap cardiac systole and diastole).

With this report we describe a procedure based on acoustic startle methodology that fully relies on pre-attentive neuronal circuitry, does not depend on conscious processing, and is linked to well-explored brainstem regions. Because it is possible to time the occurrence of the startle stimulus in millisecond resolution, variations in startle reactivity may be related to systolic and diastolic cardiac processes. The effect of cardiac cycle on the startle response has been described earlier, albeit in an interaction with mechanical stimulation of the baroreceptors (PRES methodology) (Nyklicek et al., 2005). This report will extend those earlier findings and demonstrate: (i) that startle responsiveness is affected by spontaneous cardiovascular activity, and (ii) that this effect may be absent when autonomic function is impaired.

EXPERIMENT 1

2.2 Methods

2.2.1 Participants

Thirty-eight healthy undergraduate students (26 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 6 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 (range: 19 to 30; SEM = 0.5) years. All participants provided written informed consent and were made

aware of their right to discontinue participation in the study at any time. Study procedures were approved by the local ethical committee.

2.2.2 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 Tyco Healthcare H124SG electrodes were attached below the left eye with an inter-electrode distance of 1.5 cm to assess EMG-activity of the M. 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 relationship to the participants' heartbeat served as habituation trials. EMG responses on these trials were not further analyzed. During the experimental condition 40 startle probes were presented with a jittering inter-stimulus-interval of 8 to 12 s, according to a randomized sequence: half of them were presented 230 ms after an R-wave (cardiac systole), the other half were presented 530 ms after an R-wave (cardiac diastole). The startle stimulus presentation time within the cardiac systole was based on the temporal location of (i) the arterial pulse wave appearing in peripheral blood vessels (Pitson, Chhina, Knijn, van Herwaaden & Stradling, 1994), and (ii) maximal dampening effects with respect to elicitation of peripheral reflexes (Edwards et al., 2001). Furthermore, this stimulus presentation time is in accordance with central psychophysiological effects (Leopold & Schandry, 2001) suggesting effective bottom-up transmission of cardio-afferent signals. A latency of 530 ms was defined as diastolic control (e.g., Milnor, 1990). Total length of the experimental session, including instructions, was about 10 min.

2.2.3 Recording Parameters

EMG-responses to acoustic white noise startle probes (105 dB, 50 ms duration, instantaneous rise time, binaural 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 (Biopac EMG100C) were 10 to 500 Hz, followed by software filtering (28 Hz high pass cutoff). The raw signal was rectified and integrated online with a time constant of 10 ms (Blumenthal, 1994). The ECG signal was high-pass filtered (Biopac ECG100; HPF: 0.5 Hz) and stored to disk (1 kHz) as well. R-waves were identified online by a fast DASYLAB-8.0 (National Instruments, Inc.) based algorithm running on a different CPU. Accuracy of R-wave detection in sine rhythm was higher than 99.8 %, with a latency below 3 ms (internal lab report).

2.2.4 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 to 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 artefacts, such as coinciding blinks or other facial muscular activities, were rejected from analysis and defined as missing. If responses were not visible at the typical response latency of a particular subject, response amplitude was set to zero. Zero response data were included into the averaging procedure, with startle response magnitude as the final output measure (Blumenthal et al., 2005). Averaging was done per subject and according to whether startle was elicited in the cardiac systole or diastole.

2.2.5 Statistical Analysis

A dependent *t*-test (critical α level = .05; two-tailed) was employed to statistically compare startle responses elicited during cardiac systole and diastole.

2.3 Results

All participants had a regular sinusoidal cardiac rhythm without premature heartbeats. However, two participants were excluded from analysis because of strong habituation and absence of any visible eye-blink responses during the experimental condition. Startle eye blink response magnitude was larger (T[35]= -3.94; p = .0004; η^2 = .31) when startle was elicited during diastole (M = 253 μV; SEM = 54 μV) than during cardiac systole (M = 192 μV; SEM = 44 μV).

2.4 Discussion

Our results clearly demonstrate that in young volunteers the startle eye blink response elicited by acoustic stimuli is smaller during cardiac systole than during cardiac diastole. This cardiovascular modulation of startle eye blink is in concordance with past findings indicating an attenuation of simple cognitive functions, such as reaction time (Edwards, Ring, McIntyre, Carroll, & Martin, 2007; Stewart, France, & Suhr, 2006; Weisz & Adam, 1996) and basal reflexes during the early cardiac cycle phase (Edwards et al., 2001; Nyklicek et al., 2005). These previous authors explained their findings of attenuated simple cognitive functions and basal reflexes in terms of baroreflex mechanisms, with the more intense stimulation of baro-afferents resulting in an inhibition of CNS processes. If the same baroreflex mechanism is responsible for our findings, then the cardiovascular modulation of the startle eye blink should be absent when baro-afferent signal transmission is impaired. We performed a second study to test this prediction.

EXPERIMENT 2

2.5 Methods

The experimental procedure of *Study 2* was very similar to that of *Study 1*. The main difference was the testing of diabetic patients. A long duration of diabetes often leads to autonomic neuropathy whose earliest symptom is diminished baroreflex heart rate control (Frattola et al., 1997; Gulli et al., 2005; Weston et al., 1998; Weston et al., 1996). Baroreflex heart rate control may be estimated from spontaneous beat-to-beat heart rate and systolic blood pressure changes. Baroreflex sensitivity is then calculated by transfer function analysis

(gain, transfer magnitude) (Robbe et al., 1987). Diminished baroreflex sensitivity indicates impairment of baro-afferent neuronal signal transmission.

2.5.1 Participants

Study information was mailed to Type 1 diabetes mellitus patients, regardless of individual history and co-morbidity. Patients indicating interest were then individually interviewed, as was their diabetes specialist (focusing on their diabetes treatment, diabetes complications, and psychiatric co-morbidity). Exclusion criteria included any acute uncontrolled somatic or psychiatric co-morbidity, regular use of benzodiazepines or beta-blockers, or a resting heart rate of higher than 85 bpm. It was verified that participants were on a 'state of the art' intensified insulin regimen, performed 3-5 insulin injections and at least 3 blood glucose measurements per day, and had a recent adjustment of insulin dose and dosing schedule (if necessary). Thirty-nine patients (18 women) qualified to participate in the experiment. Similar to *Study 1*, participants provided written informed consent and were made aware of their right to cancel participation in the study at any time. Study procedures were approved by the local ethical committee.

2.5.2 Procedure

All examinations were scheduled in the morning during routine hours. Diabetes patients were seated in a comfortable armchair. Electrodes for measurement of ECG and EMG-activity of the M. orbicularis oculi, as well as headphones, were attached according to *Study 1* methods described above. Additionally, non-invasive continuous blood pressure (Finapres system, Ohmeda, Englewood, USA) was recorded. Cardiovascular beat-to-beat data were assessed during a 5-min resting period during which subjects were instructed to close their eyes, relax, and neither move nor speak.

After the resting period patients remained seated. Finapres was detached to avoid any somato-sensory feedback of cardiac activity. After a 3-min break, six startle probes (see above: *Study 1*) were delivered via headphones for purposes of early startle habituation. Then patients listened to a train of 25 startle probes (inter-stimulus-interval randomly ranging between 8

and 12 s), which were delivered without any contingence to R-waves. Parameters of the acoustic white noise startle probes were identical to those of *Study 1*. Startle presentation was part of a more extensive study protocol and not the only purpose of the study. Thus, the 25 startle probes were not linked to cardiac activity and were expected to be randomly distributed over the whole cardiac cycle.

2.5.3 Recording Parameters

The recording settings were matched with those of *Study 1* (amplification, sampling, data conditioning, with the exception of the integrator time constant = 20 ms). The ECG signal, an analog marker signal, the raw EMG, and the hardware-filtered EMG signal were digitally converted (12 bit) and stored on hard disk for offline analysis. All cardiovascular signals were digitally converted (12 bit, 1 kHz) and stored on disk. Inter-beat-interval and beat-to-beat blood pressure data were then calculated offline. Beat-to-beat data were manually edited for outliers due to artifacts or premature ventricular and supraventricular myocardial activity as follows: all beat-to-beat ECG data were displayed on a computer screen by proprietary software. If automatic beat detection (triggering on the R-wave) did not reveal the correct time points due to electrical artifacts (0.2% of all beats), triggering on the P-wave (0.05% of all beats), or triggering on the T-wave (0.01% of all beats), data were corrected by computer-assisted manual control. Ventricular ectopic beats are characterized by a changed QRS morphology, and were identified by visual inspection of all beat-to-beat ECG data. They occurred with a frequency of 0.7% in the final study sample, but never in adjacent beats. They were handled by simple interpolation of the two affected inter-beat-intervals (sum of both intervals, divided by two). This guaranteed proper alignment of the following beat data. A similar procedure was used to control for supraventricular premature beats, which are characterized by an altered P-wave morphology, and occurred with a frequency of 0.3% of all beats.

Baroreflex sensitivity (BRS) of cardiac chronotropic control was assessed by employing the transfer function analysis routine of the CARSPAN program (Mulder, 1995). CARSPAN's algorithm of transfer function analysis is based on discrete Fourier transformation of non-equidistant samples of systolic blood pressure (input) and inter-beat-interval (output) pairs of various series lengths. CARSPAN provides a uniform output of 0.01 Hz resolution for periods

longer than 100 s. Transfer functions (coherence and transfer magnitude) were determined within the 0.07 to 0.14 Hz band. Baroreflex sensitivity was calculated by integrating the transfer magnitude (modulus function) over frequency points with coherence values higher than 0.5 (Robbe et al., 1987). CARSPAN has previously been successfully applied to study baroreflex sensitivity in diabetic patients (Lefrandt et al., 1999). The total sample was divided into two groups according to one of the baroreflex sensitivity risk stratification cutoffs identified to predict cardiac mortality in the ATRAMI study (La Rovere et al., 1998), having diminished (< 6 ms/mmHg) or near normal (> or = 6 ms/mmHg) BRS of cardiac chronotropic control. In diabetes studies, this cutoff value is furthermore justified as it allows for an optimal discrimination between control and diabetes- related organ damage (Lefrandt et al., 1999).

2.5.4 Startle Response Analysis

Startle responses were analyzed according to similar standards as in *Study 1*. All responses were classified as to whether elicitation occurred during cardiac systole (100 to 400 ms after the R-wave), or diastole (401 to 800 ms after the R-wave). A minimum of 5 startle responses had to be present during cardiac systole and diastole for that participant's data to be included in further analysis.

2.5.5 Statistical Analysis

Individually averaged startle response data were subject to a mixed-design ANOVA to examine differences between startle responses elicited during cardiac systole vs. diastole (within-subjects factor) in patients with lower or higher BRS than 6 ms/mmHg (between-subjects factor), and the interaction term. All testing was two-tailed. Statistical calculations were performed using SAS (Version 9, WinXP, SAS Institute, Cary, NC, USA).

2.6 Results

Five patients either did not have a normal sinusoidal cardiac rhythm, had resting heart rate above 85 bpm, or had more than six premature ventricular ectopic heartbeats per minute, and were thus excluded from further analysis. Two patients showed a complete startle habituation

and were not considered for further analysis, as was one patient with insufficient frequency of startle responses elicited during cardiac systole. Data of 31 patients remained.

2.6.1 Group Division via Baroreflex Sensitivity

Thirteen diabetes patients (5 women) had a BRS lower than 6 ms/mmHg. Mean BRS of this group was 2.9 (SEM = 0.3) ms/mmHg, age was 44 (SEM = 3.0) years, diabetes duration was 28 (SEM = 2.1) years, resting blood pressure was 144 (SEM = 8) / 73 (SEM = 4) mmHg, and mean heart rate was 68 (SEM = 3) bpm. Eighteen patients (7 women) had a BRS higher than 6 ms/mmHg. Mean BRS of this group was 9.6 (SEM = 0.7) ms/mmHg, age was 39 (SEM = 4.0) years, diabetes duration was 11 (SEM = 1.2) years, resting blood pressure was 140 (SEM = 4) / 77 (SEM = 2) mmHg, and mean heart rate was 72 (SEM = 2) bpm. Statistical analysis (group T-test) revealed a significant difference of diabetes disease duration (T[28] = 7.14; p = .0001). The groups did not differ in age (T[28] = .94; p = .34), nor in cardiovascular variables such as heart rate (T[28] = 1.10; p = .28), systolic (T[28] = .54; p = .59) and diastolic blood pressure (T[28] = .79; p = .44). None of the diabetes subjects suffered from acute vascular disease (i.e., coronary heart disease, cerebral ischemia), kidney failure, gastroparesis, or obesity. Metabolic control was similar between both groups, as suggested by almost identical group mean data for glycosylated haemoglobin (HbA_{1c}, immuno-enzymatic method, upper limit of normal range: 6.1-6.3%; low BRS: M = 6.65%; SD = .95; normal BRS: M = 6.76%; SD = 1.03; T[28] = .26; p = .79), mean blood glucose values (low BRS: M = 8.2 mmol/l; SD = 1.2; normal BRS: M = 8.4 mmol/l; SD = 1.8; T[28] = .49; p = .62) and daily insulin dosage (low BRS: M = 46 units; SD = 8; normal BRS: M = 40; SD = 8; T[28] = 1.88; p = .07).

2.6.2 Startle Responses

In the group with BRS higher than 6 ms/mmHg, startle eye blink response magnitude was diminished during cardiac systole (M = 137 µV; SEM = 6 µV) as compared to during cardiac diastole (M = 170 µV; SEM = 5 µV). This effect did not appear in the low BRS group (see Figure 1) where mean eye blink response magnitude was unaffected by cardiac cycle phase (systole: M = 156 µV; SEM = 5 µV; diastole: M = 160 µV; SEM = 5 µV). ANOVA revealed a significant interaction (F[1,29] = 5.91; p = .02; η^2 = .17) between the group factor (BRS < 6 vs. BRS > 6 ms/mmHg) and within-subjects factor (cardiac systole vs. diastole). The main

within effect of cardiac cycle phase was also significant (F[1,29] = 9.46; p = .004; η^2 = .25), but not the main group effect (F[1,29] = 1.25; p = .27). Including sex as a covariate in the statistical model favoured the group × cardiac cycle phase interaction (F[1,28] = 6.66; p = .015; η^2 = .19). Over the whole group of 31 patients, BRS and the difference in startle response magnitude between systole and diastole were significantly correlated (r = .45; p = .02). Startle magnitudes reported here were smaller than in *Study 1*. This may be due to a slightly longer time constant of integration, which can attenuate response magnitude (Blumenthal, 1994), and to a previously described effect of age on startle responsiveness (Ford et al., 1995) .

Within the low BRS group the mean of valid and analyzed startle responses during the cardiac systole was 8.2 (SD = 1.9; mean latency = 240 ms), during the cardiac diastole a mean of 12.5 (SD = 2.4) startle responses were analyzed (mean latency = 599 ms). Numbers of analyzed startle responses within the normal BRS group: cardiac systole: M = 9.6 (SD = 3.0; mean latency: 254 ms); cardiac diastole: M = 11.6 (SD = 2.7; mean latency: 634 ms).

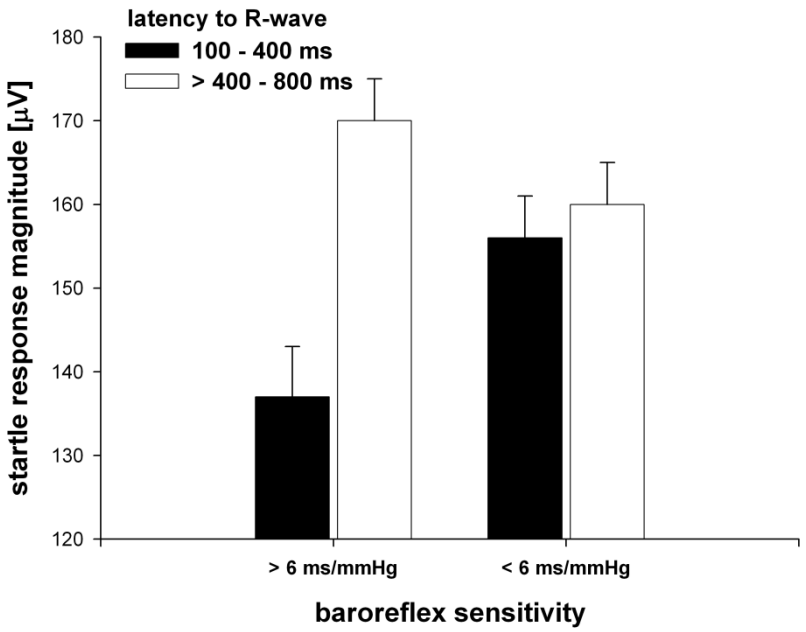

Figure 1. Startle response magnitude in response to acoustic stimulation elicited 100 to 400 ms after the R-wave (cardiac systole) and after 400 ms (diastole) in two groups of diabetic patients, characterized by baroreflex sensitivity of heart rate control lower (n=13) or higher (n=18) than 6 ms/mmHg.

2.7 General Discussion

Two studies are reported. The first study demonstrated that acoustic startle response magnitude is modulated according to whether startle responses were elicited during cardiac systole or diastole. The second study replicated the result of the first study and, furthermore, demonstrated that intact baroreflex heart rate control may be essential for this effect.

Baroreflex heart rate control relates to a mechanism of counter-balancing heart rate in response to blood pressure changes. The neural pathways of this reflex mechanism are well described (Jänig, 2006). The afferent component originates from baroreceptors located in the carotid sinus and aortic arch that project to the Nucleus tractus solitarius (NTS) via the glossopharyngeal and vagal cranial nerves. The parasympathetic output branch involves the Nucleus ambiguus (NA), Dorsovagal motor nucleus, and vagal efferents, whereas the slower sympathetic output branch involves the Rostroventrolateral medulla (RVLM), as well as pre- and post-ganglionic sympathetic efferent neurons. Autonomic diabetic neuropathy is known to affect afferent as well as efferent neural structures (Fazan, Salgado & Barreira, 2006; Salgado, Fazan Junior, Fazan, Da Silva & Barreira, 2001) with the result of impaired baroreflex function. Indeed, impaired baroreflex sensitivity is one of the earliest signs of diabetic autonomic nervous system pathology (Frattola et al., 1997; Gulli et al., 2005; Weston et al., 1998; Weston et al., 1996).

Our second study shows that disturbed baroreflex heart rate control was associated with a loss of cardiac modulation of startle. To our knowledge, the nuclei operating vagal and thoracic sympathetic-neural output (e.g. NTS, NA, RVLM) do not connect with the somatic startle center (Nucleus reticularis pontis caudalis), nor with motor output structures involved in the eye blink (e.g., Facial nucleus in the brainstem) (Davis et al., 1999), so do not play a direct role in startle eye blink responding. Thus, it is justified to assume that impairment of baro-afferent signal transmission is responsible for the missing cardiac modulation of startle in diabetic autonomic neuropathy.

Several other baroreflex mediated effects on higher CNS processes have been described. Association of elevated blood pressure and reduced sensitivity to physically aversive

stimulation was reported first by (Dworkin et al., 1979) in an animal study, and was replicated in human hypertension by applying electrical (Zamir & Shuber, 1980), thermal (Rau et al., 1994; Sheps et al., 1992) and tactile painful stimulation (Bruehl et al., 1992). Natural stimulation of baroreceptors across the normal cardiac cycle alters pain perception and affects other central and peripheral functions (Edwards et al., 2003; Edwards et al., 2002; Edwards et al., 2001; Edwards et al., 2007; Stewart et al., 2006; Weisz & Adam, 1996), such as EEG theta band activity (Rau et al., 1993), and prolongs reaction time in simple tasks (Edwards et al., 2007; Stewart et al., 2006; Weisz & Adam, 1996).

Unfortunately, it is still impossible to selectively stimulate baro-afferents in human 'in vivo' studies. However, the phase related external suction (PRES) technique (Rau et al., 1994; Rau et al., 1992) has been used to amplify baro-afferent effects mechanically, by using a neck cuff which generates a negative pressure by suction to increase the diameter of the carotid sinus and stimulate baroreceptors as during an arterial pulse wave. In contrast, inflating the cuff causes decreasing diameter of arteries comparable to that during cardiac diastole, and unloads baroreceptors. Negative cuff pressure is usually elicited during the early cardiac cycle phase, when natural stimulation of baroreceptors is at maximum; positive pressure is elicited during late cardiac cycle phase to minimize their stimulation. This paradigm results in a general attenuation of startle magnitude during the first half of the cardiac cycle compared with the second half, where startle response was higher (Nyklicek et al., 2005). PRES always induces collateral somato-sensory and acoustic stimulation that may cause attentional modulation of startle responsiveness. Furthermore, because PRES is time-locked with cardiac activity, it may induce a bias based on potential prepulse inhibition (PPI) effects, wherein a stimulus presented just before a startle stimulus inhibits the response to that startle stimulus (Blumenthal, 1999). However, because the study cited above resulted in a significant interaction effect of mechanical procedures and cardiac cycle, PPI by PRES cannot explain the whole effect. Interestingly, even without stimulation, heartbeat perception could induce a PPI effect, since almost any stimulus can act as a prepulse (Blumenthal, 1999). Cardiac perception involves the somatosensory modality (Jones, Jones, Rouse, Scott & Caldwell, 1987) and chest wall afferents may be activated by cardiac vibrations (Knapp, Ring & Brener, 1997), and sounds may also be induced by the cardiac systole (Velluti, Pena, Pedemonte & Narins, 1994). In any case, heartbeats are found to have a saliency near the sensory threshold in most subjects (Ring & Brener, 1992) and it is known from neuroimaging studies that

heartbeat signals reach higher brain areas beyond the brainstem. In this regard, activity of right Anterior insula (AI) was found during pharmacologic stimulation of baroreceptors via α_1-adrenergic medication (Henderson et al., 2004). This area is also considered to be involved in processes of cardiac perception and awareness. Activity and total gray matter volume of AI correlate with cardioceptive accuracy (Critchley et al., 2004). This suggests that baro-afferent feedback and cardiac perception, which may be transmitted over visceral-afferent pathways, share neuronal convergences. Whether these processes have the potential of causing a PPI effect remains unknown, but future studies should address this issue.

In the research literature, there is no consensus about fixed timing intervals that separate early and late cardiac cycle phases. Some studies used the recent inter-beat intervals to predict the length of current cardiac cycle phase to differentiate early and late phases (Nyklicek et al., 2005). Others used fixed intervals of 0, 300, and 600 ms for early, middle, and late cardiac cycle phase (Edwards et al., 2007). Nevertheless, it can be argued that a varied stimulus interval between 100 and 400 ms after the R-wave can be defined as the early cardiac cycle phase that is associated with heightened systolic arterial blood pressure (Milnor, 1990). In *Study 1*, startle probes were triggered by delays of either 230 or 530 ms after the actual ECG-recorded R-wave. By contrast, in *Study 2*, delays between the R-wave and startle stimulus presentation (systole: 100 to 400 ms; diastole: > 400 ms) were calculated post-hoc. This allowed for a more homogeneous distribution of startle elicitations over the cardiac cycle. However, the timing of startle stimuli in *Study 1* is supported by previous reports indicating dampening of the nociceptive flexion response (Edwards et al., 2001). Moreover, electrocortical potentials (Leopold & Schandry, 2001) and simultaneous judgments of heartbeats and exteroceptive signals (Ring & Brener, 1992; Ring, Liu & Brener, 1994) suggest effective bottom-up transmission of cardio-afferent signals at this latency. Accordingly, we presume that a delay of 230 ms (as used in *Study 1*) is associated with CNS representation of visceral-afferent input originating from the heartbeat. In contrast, about 300 ms later, at a delay of 530 ms, stimulation of baroreceptors should tend to be minimal (e.g., Mancia & Mark, 1983).

2.7.1 Limitations

We have used somewhat different startle timing strategies and hardware settings in *Study 1* and *Study 2*, due to the fact that both studies were initially planned for different purposes. However, both studies incorporated their own control conditions, and data from the two studies are not analyzed in the same statistical model. Results of *Study 2* are based on fewer startle stimulations, as compared to *Study 1*. A replication study with identical startle methods of the volunteer and diabetic study parts would be desirable.

Although a dependency of intact baro-afferences on the cardiac modulation effect of startle eye blink may be assumed, the quasi-experimental design of *Study 2* with two groups of diabetic patients does not allow for a conclusion of causality.

2.7.2 Conclusion

Our results indicate that startle response magnitude is affected by cardiac cycle phase, and that baro-afferent signal transmission is associated with this effect. This effect may prove useful to explore baro-afferent integration, since it is based on pre-attentive processing and background stimulation, and does not require active involvement by the participant. In this respect, the use of acoustic startle probes has the advantage of a very short timeframe between stimulation, modulation, and response; therefore, startle methodology has the potential to investigate very rapid processes (Bradley et al., 1991; Koch, 1999). A further implication is that future startle research should consider controlling for this effect when cardiovascular changes are to be expected.

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2.ii AUTHOR NOTES

Address reprint requests to: Hartmut Schächinger, Institute of Psychobiology, University of Trier, Johanniterufer 15, D-54290 Trier, Germany. E-mail: schaechi@uni-trier.de.

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With deep regret, we need to report that our decent friend and colleague Willi Berger died during the preparatory phase of this manuscript

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Chapter III:

Lateralization Effects on the Cardiac Modulation of Acoustic Startle Eye Blink

(Schulz et al., 2009b)

Co-Authors: Johanna Lass-Hennemann, Steffen Richter, Sonja Römer, Terry D. Blumenthal & Hartmut Schächinger.

3.0 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 viscero- and baro-afferent signal transmission.

Keywords: Baroreflex, Hemispheric Specialization, Interoception, Lateralization, Startle Modulation, Visceral Afferents

3.1 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, 1981a) 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., 2009a). 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., 2009a).

It is well described that central processing of active heartbeat perception (Critchley et al., 2004; Pollatos et al., 2007b) 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 viscero- 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 & 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 M. orbicularis oculi (Bradley et al., 1991; Bradley et al., 1996; Hackley & Graham, 1987; Kettle, Andrewes & Allen, 2006; Kofler, Muller, Rinnerthaler-Weichbold & Valls-Sole, 2008), whereas generally facilitated responsiveness with right-ear stimulation is less consistently found (Hackley & 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 & Nachshon, 1973; Haggard & 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., 2009a) 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.

3.2 Methods

3.2.1 Participants

Fourty-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 to 29; SD = 2.5) years. Mean heart rate was 76.6 (SD = 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 eye blink 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 (SD = 2.4) years and a mean resting heart rate of 70.5 (SD = 8.8) bpm. All participants had a regular sinusoidal cardiac rhythm without premature heartbeats. Study procedures were approved by the local ethical committee.

3.2.2 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 M. 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 contingence 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 to 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.

3.2.3 Recording Parameters

EMG-responses of the left and the right M. 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 Koden EEG 4421G pre-amplifier) were 0.5 to 500 Hz, followed by software filtering (28 Hz high pass cutoff: van Boxtel, Boelhouwer & Bos, 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 ECG100, HPF: 0.5 Hz) and stored to disk (1 kHz) as well. R-waves were identified online by a fast DASYLAB-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).

3.2.4 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 to 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% (SD = 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 μV, 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).

3.2.5 Statistical Analysis

A 2 × 2 × 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 α-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.

3.3 Results

Our analysis within the ANOVA model indicated no significant main effect for the presentation side (left: M = 111 μV; SEM = 21 μV; right: M = 113 μV; SEM = 21 μV; $F[1,36] < 1$), nor for the side of measurement (left: M = 111 μV; SEM = 23 μV; right: M = 114 μV; SEM = 21 μV; $F[1,36] < 1$). However, the interaction of presentation side × 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 μV; SEM = 25 μV; right: M = 126 μV; SEM = 23 μV) than in contralateral presentation (left: M = 101 μV; SEM = 19 μV; right: M = 101 μV; SEM = 20 μV) (see Figures 2 and 3).

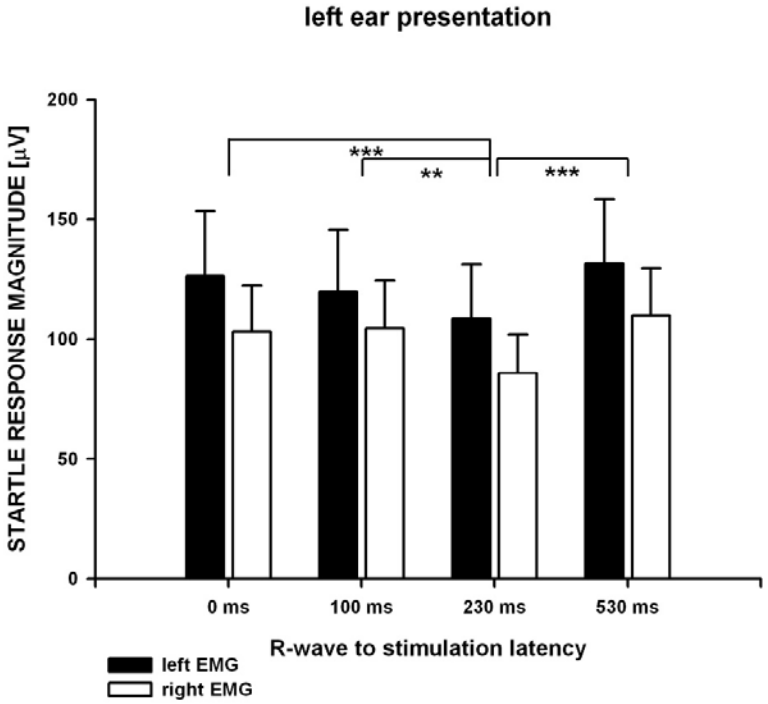

Figure 2. At a latency of 230 ms, the startle response magnitude is significantly attenuated for left ear stimulation, independent of the side of measurement. The absence of modulation effects at earlier latencies supports the recent thesis that baro-afferent neural traffic is responsible for the modulation (error bars: SEM).

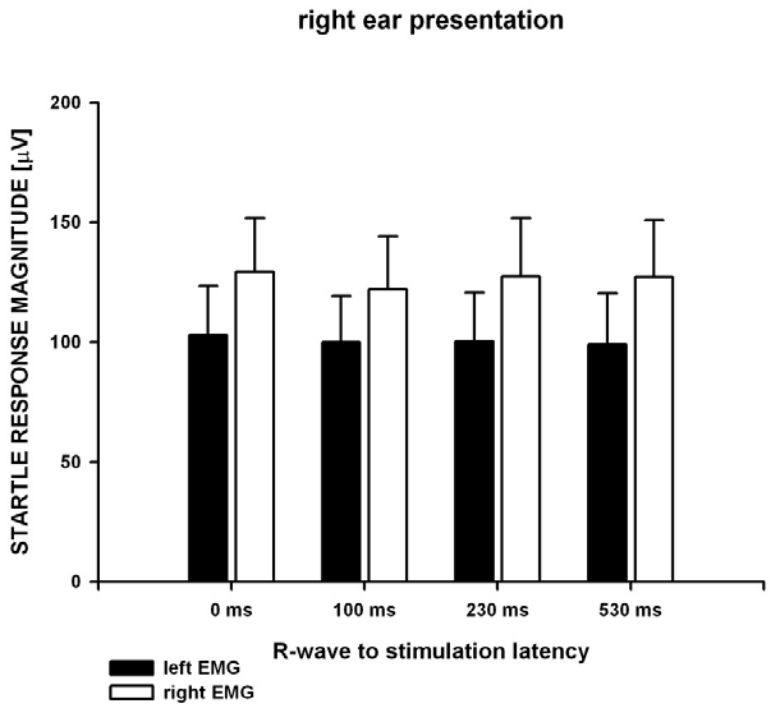

Figure 3. At right-ear presentation, no main effect of R-wave latency was detected. Note the interaction of presentation side × side of measurement (compare Figures 2 and 3), illustrating an ipsilateral advantage.

Replicating recent results (Schulz et al., 2009a), 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 μV; SEM = 20 μV), compared to the latencies of 0 ms (M = 115 μV; SEM = 22 μV), 100 ms (M = 111 μV; SEM = 21 μV), and 530 ms (M = 117 μV; SEM = 22 μV). 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 × cardiac cycle (F[3,108] = 4.609; $p < .01$; $\eta^2 = .113$). This result suggests an appearance of the cardiac modulation effect only if acoustic startle stimuli were presented to the left ear. A post-hoc analysis indicated that this effect was present in the startle response magnitude that was

assessed at the left and at the right side (see Figures 2 and 3). When the four latency conditions were evaluated for left side stimulation and left side measurement (F[3,108] = 6.477; $p < .001$; $\eta^2 = .152$) reactivity in the R-wave +230 ms condition (M = 109 μV; SEM = 23 μV) differed significantly from that in the +0 ms (M = 126 μV; SEM = 27 μV; $p = .017$), +100 ms (M = 120 μV; SEM = 26 μV; $p = .046$), and +530 ms (M = 132 μV; SEM = 27 μV; $p < .001$) conditions. When the four latency conditions were evaluated for left side stimulation and right side measurement (F[3,108] = 8.969; $p < .001$; $\eta^2 = .199$) the +230 ms condition (M = 86 μV; SEM = 16 μV) differed from the +0 ms (M = 103 μV; SEM = 19 μV; $p < .001$), +100 ms (M = 104 μV; SEM = 20 μV; $p < .005$), and +530 ms (M = 110 μV; SEM = 20 μV; $p < .001$) conditions (see Figure 2). In contrast, when the four latency conditions were evaluated for right side stimulus presentation, neither the simple main effect of latency of left-sided EMG assessment (0 ms: M = 103 μV; SEM = 21 μV; 100 ms: M = 100 μV; SEM = 19 μV; 230 ms: M = 100 μV; SEM = 20 μV; 530 ms: M = 99 μV; SEM = 21 μV), nor the simple main effect of latency of right-sided EMG assessment (0 ms: M = 129 μV; SEM = 23 μV; 100 ms: M = 122 μV; SEM = 22 μV; 230 ms: M = 127 μV; SEM = 25 μV; 530 ms: M = 127 μV; SEM = 24 μV) revealed an effect of the cardiac cycle on the startle response magnitude (see Figure 3). We conclude that the cardiac modulation effect of startle eye blink is only present in left-sided startle stimulation, regardless of the side of EMG startle response assessment.

3.4 Discussion

The aims of our study were to investigate whether cardiac modulation of startle eye blink depends on left vs. right ear acoustic startle stimulus presentation side, and to test whether left vs. right side eye blink measurements are differentially affected by cardiac influences. Further aims were to investigate whether cardiac modulation of startle eye blink appears very early in the cardiac cycle (e.g., at 0 ms and 100 ms latencies after adjacent R-waves), and to replicate the previous finding of cardiac modulation of startle eye blinks at 230 ms latency after adjacent R-waves. The main findings of this study are that cardiac modulation of acoustic startle is present at 230 ms latency after the R-wave following left ear stimulation only, and that the side of measurement does not play a role in this effect.

This is the first monaural acoustic noise stimulation study to confirm that startle eye blinks may be influenced by cardiac cycle time during spontaneous cardiac resting activity. This effect was most prominent at a latency of 230 ms after the R-wave, and did not occur at earlier latencies within the cardiac cycle. The timing of this effect is in concordance with earlier results in which maximal attenuation of basal reflexes (Edwards et al., 2001; Schulz et al., 2009a) were found at a latency of 230 ms after adjacent R-waves, and thus, supports the hypothesis that baro-afferent neurotransmission is responsible for this effect (Nyklicek et al., 2005; Schulz et al., 2009a). However, this does not necessarily mean that the temporal location of this effect may not change under conditions of activation of the autonomic nervous system. The concordance between the present study and a recent study (Schulz et al., 2009a) may be due to the fact that all participants were tested during resting conditions only. Autonomic activation, especially via physical stress, is known to raise heart rate (Lovallo, 1975; Sherwood, Allen, Obrist & Langer, 1986), shorten pre-ejection period (Schachinger et al., 2001), and shorten pulse transit time (Weiss, Del Bo, Reichek & Engelman, 1980). Therefore, participants with a high level of autonomic activation are expected to show a similar pattern of cardiac modulation, but with an earlier attenuation of the startle response within the cardiac cycle. Future studies should address this question.

We found an interaction between side of acoustic noise presentation and side of eye blink measurement, with increased startle eye blink response magnitude at the ipsilateral measurement side during monaural acoustic noise presentation. This is in concordance with past studies (Bradley et al., 1991; Bradley et al., 1996; Hackley & Graham, 1987) indicating that the ipsilateral M. orbicularis oculi is more rapidly and more intensively activated (Hackley & Graham, 1987). This ipsilateral advantage is the product of sensory and motor crossing, and it is safe to speculate that this hardwiring guarantees fast and error-free eye protection at the side of exposure, much faster than conscious source location during choice reaction time procedures may allow for. Thus, although several previous studies indicated acoustic information processing to be much less lateralized than visual processing, and although each motor nucleus innervates both M. orbicularis oculi (Kettle et al., 2006), this and other startle studies suggest that noise processing is lateralized with distinct startle-eliciting sensory information crossing to the contralateral side.

The principal new finding of this study was the restriction of cardiac modulation of startle to acoustic noise probes which were presented to the left ear, independently of the side of measurement. Given left ear to right hemisphere sensory information crossing, and the fact that visceral-afferent and baro-afferent signals are processed primarily in the right hemisphere, the observed left ear modulation effect may reflect the advantage of combining both neural processes in the same hemisphere. This assumption is supported by two kinds of neuro-imaging studies, one focusing on interoceptive awareness (Critchley et al., 2004; Pollatos et al., 2007b), and the other focusing on baroreceptor stimulation effects (Henderson et al., 2004; Kimmerly et al., 2005). Both kinds of studies identified similar cortical brain regions (e.g., right Anterior insula, Anterior cingular cortex), strongly suggesting that these areas play a role in viscero-afferent signal processing. However, it remains unclear whether the cardiac modulation of startle eye blink depends on any cortical structure at all. Given that the baroreflex (Jänig, 2006) and the primary acoustic startle circuit (Davis et al., 1999; Koch, 1999) are both brainstem reflexes, it is probable that higher brain structures play only a limited role in this modulation. Interestingly, a similar left side lateralization effect has been found in affective startle research (Bradley et al., 1991; Bradley et al., 1996; Kettle et al., 2006). Human startle augmentation and inhibition may be induced by complex appetitive and aversive visual foreground materials, the processing of which clearly requires some higher cortical central nervous system competence. It was suggested that the lateralization effect in the affective startle paradigm is caused by a right-hemispheric advantage in processing affectively relevant stimuli (Bradley et al., 1991; Bradley et al., 1996). The modulation of fear-potentiated startle in a rodent model is due to Amygdala input to the startle center (the Nucleus reticularis pontis caudalis (Davis, 2006; Davis et al., 1999; Frankland, Scott & Yeomans, 1995). Given that the right temporal lobe, although being closely connected with the left Amygdala (Kettle et al., 2006), was found to be responsible for affective modulation of startle in humans (Angrilli et al., 1996; Funayama et al., 2001), one may speculate that cardiac modulation of startle eye blink may also be affected by Amygdalae activity. Indeed, interoceptive neural traffic was found to reach the Amygdala (Cechetto & Calaresu, 1984; Cechetto & Calaresu, 1985) via the Nucleus tractus solitarius (Jänig, 2006). Thus, the presence of anatomical connections suggests that the Amygdalae have the potential to influence the cardiac modulation of startle and its lateralization.

A generally higher startle responsiveness of the right M. orbicularis oculi muscle was found by Hackley and Graham (1987), Grillon and Davis (1995), and Kofler et al. (2008), but not in this study. However, this effect may potentially be influenced by handedness (Kofler et al., 2008). Although this effect was particularly found in right-handed subjects, who were also investigated in the current study, it could not be replicated here. Furthermore, the described lateralization effect of cardiac modulation of startle eye blink may also depend on subjects' handedness, which was not controlled in this study.

3.4.1 Limitations

Several limitations need to be acknowledged. Only monaural acoustic stimulations were applied, to allow evaluation of this study in the context of several earlier startle studies which used monaural startle stimulation to explore lateralization effects (Bradley et al., 1991; Bradley et al., 1996; Grillon & Davis, 1995; Hackley & Graham, 1987; Kofler et al., 2008). However, the distinct stimulation of only one ear may be an uncommon condition that does not have a natural analog. Thus, lateralization effects found with this methodology should be interpreted with some caution. Furthermore, in this study we did not include a control condition with binaurally presented startle. However, the effects of bilateral startle presentation across the cardiac cycle were already reported (Schulz et al., 2009a), but not for earlier latencies (R +0 ms; +100 ms). This study clearly shows that laterality plays a role in cardiac modulation of startle eye blink. It was not the purpose of this study to identify stimulus characteristics, intensities, and latencies which will lead to maximal effectiveness of the laterality factor, although all of these should be assessed by future research.

3.4.2 Conclusion

Cardiac modulation of acoustic startle eye blink, which is associated with baro-afferent neural feedback, depends on stimulus timing and the side of acoustic stimulus presentation. The attenuation of startle responsiveness occurred at a latency of 230 ms after the R-wave, and after left ear stimulus presentation only. This lateralization effect in the cardiac modulation of startle eye blink may reflect the advantage of right-hemispheric brain structures in processing viscero- and baro-afferent neural signals.

3.i REFERENCES – Chapter III

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3.ii AUTHOR NOTES

Please correspond to: André Schulz, Dipl.-Psych., Institute of Psychobiology, University of Trier, Johanniterufer 15, D-54290 Trier, E-Mail: andre.schulz@uni-trier.de.

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Chapter IV:

Cardiac Modulation of Startle: Effects on Eye Blink and Higher Cognitive Processing

(Schulz et al., 2009c)

Co-Authors: Carolin F. Reichert, Steffen Richter, Johanna Lass-Hennemann, Terry D. Blumenthal & Hartmut Schächinger.

4.0 Abstract

Cardiac cycle time has been shown to affect pre-attentive brainstem startle processes, such as the magnitude of acoustically evoked reflexive startle eye blinks. These effects were attributed to baro-afferent feedback mechanisms. However, it remains unclear whether cardiac cycle time plays a role in higher startle-related cognitive processes, as well. Twenty-five volunteers responded first by 'fast as possible' button pushes (reaction time, RT), and second, rated perceived intensity of 60 acoustic startle stimuli (85, 95, or 105 dB; 50 ms duration; binaural; instantaneous rise time), which were presented either 230 or 530 ms after the R-wave, and eye blink responses were measured by EMG. RT was divided into evaluation and motor response time according to previous research. Increasing stimulus intensity enhanced startle eye blink, intensity ratings, and RT components. Eye blinks and intensity judgments were lower when startle was elicited at a latency of R +230 ms, but RT components were differentially affected: the evaluative component was attenuated, and the motor component was accelerated when stimuli were presented 230 ms after the R-wave. We conclude that the cardiac cycle affects the attentive processing of acoustic startle stimuli.

***Keywords*:** Baroreflex; Cardiac Cycle; Intensity Judgments; Interoception; Movement Time; Reaction Time; Startle Modulation; Stimulus Evaluation; Visceral Perception

4.1 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). Cardiac modulation of startle eye blink (CMS) has been established as a 'background' methodology to assess cardio-afferent traffic, since it relies on intact baro-afferents and does not require active cooperation from participants (Schulz et al., 2009a; Schulz et al., 2009b). In this paradigm, the increased baro-afferent feedback during the early cardiac cycle phase attenuates startle responsiveness compared with the late cardiac cycle phase. Neural circuits for both startle (Davis, 2006) and the baroreceptor reflex (Jänig, 2006) have been identified and found to be relayed at the level of the brainstem, with only three synapses in each circuit. However, it is yet unclear whether the pre-attentional modulation of startle responses during the early cardiac cycle phase is also reflected by an altered cognitive evaluation of these eliciting stimuli.

In earlier studies, the impact of startle on parameters of cortical signal processing was substantiated by the finding that stimulus preferences of startling noises may be reflected by different response patterns in the electroencephalographic P300 component (Davis & Heninger, 1972; Hirano, Russell, Ornitz & Liu, 1996; Putnam & Roth, 1990), which indicates a cognitive evaluation of the incoming stimulus. In another common pre-attentive startle modulation paradigm, the prepulse inhibition (PPI) of startle responsiveness (Blumenthal, 1999), in which weak pulses prior to the startle noise attenuate the startle response, the EMG responsiveness converges with subjectively perceived intensity (Blumenthal, Schicatano, Chapman, Norris & Ergenzinger, 1996; Swerdlow et al., 2007; Swerdlow et al., 1999; Swerdlow et al., 2005). To our knowledge, baroreflex-modulated reflex responsiveness is neither reflected by subjective aversiveness ratings of the eliciting stimulus itself (Edwards et al., 2003; Edwards et al., 2001), nor by ratings of emotional pictures that are presented within different cycle phases (Nyklicek et al., 2005), although baro-afferent traffic has been found to reach higher cortical structures, such as the right Anterior insula in the Temporal cortex (Henderson et al., 2004; Kimmerly et al., 2005).

The simple response time to a startling stimulus may represent another measure for assessing higher cortical processing. Response times to non-startling stimuli are known to be affected

by a long-term increase of baroreceptor loading, as was found in hypertensive patients (Harrington et al., 2000; Karla et al., 1993) and by phasic variation of baroreceptor simulation across the cardiac cycle (Stewart et al., 2006; Weisz & Adam, 1996). A distinction between the stimulus evaluation component and the motor execution component of the response time revealed that the evaluation component is more likely responsible for the prolongation effect (Edwards et al., 2007).

However, the startle reflex is a defensive reflex that prepares the organism for an evasive motor response (Koch, 1999). A more intense startle stimulus shortens sensorimotor response time, regardless of whether the startle stimulus itself is the go-stimulus (Carlsen et al., 2007) or it is presented together with a go-stimulus (Lipp, Alhadad & Purkis, 2007; Lipp, Kaplan & Purkis, 2006; Valls-Sole et al., 1995). Thus, we assume that a dampening effect of baroreceptor feedback on startle responsiveness may impair both the evaluation and the motor component of the response time, if the go-stimulus is a startle-eliciting stimulus.

To evaluate the potential cortical impact of cardiac modulated startle we conducted a within-subject experiment with 25 healthy volunteers who received 60 randomized acoustic startle probes. The startle probes had an intensity of 85, 95, and 105 dB(A) and were presented with a latency of 230 ms and 530 ms after the participants' R-wave, identical to an earlier study protocol (Schulz et al., 2009a). The definition of R +230 ms as the early cardiac cycle phase was based on the fact that (i) at this delay relative to the R-wave reflex responsiveness is maximally attenuated by baroreceptor feedback (Edwards et al., 2001), suggesting that neural output of arterial baroreceptors is at maximum around R +230 ms (Edwards et al., 2009), and (ii) simultaneous judgments of heartbeats and exteroceptive signals (Ring & Brener, 1992; Ring et al., 1994) suggest effective bottom-up transmission of cardio-afferent signals at this latency. At R +530 ms, the neural baro-afferent traffic should be minimal (e.g., Mancia & Mark, 1983; Milnor, 1990). The participants were asked to hold a home button pressed until they heard a startle sound, then to release it and to press a response button as fast as possible. After that they rated the subjective intensity of the startle sound. This procedure may clarify whether the cardiac modulation effect of startle is also reflected by modulation of cognitive evaluation processes such as stimulus evaluation and simple response times.

4.2 Method

4.2.1 Participants

Twenty-five young healthy undergraduate students (fourteen women) 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 six months, medication other than occasional pain killers and oral contraceptives, confirmed somatic or psychiatric diseases within the last six months other than banal infections or minor injuries, or a BMI lower than 19 or higher than 25 kg/m², were exclusion criteria. One individual was rejected before data collection due to acute psychopharmacologic medication. Furthermore, we excluded potential participants with a resting heart rate of higher than 85 bpm to prevent startle stimuli from extending into the next cardiac cycle. Mean age of the participants was 25.2 (range: 19-30; SD = 4.1) years, mean resting heart rate was 75.6 (SD = 7.1) bpm, and mean BMI was 21.4 (SD = 1.4) kg/m². All participants gave their written informed consent and were made aware of their right to discontinue participation in the study at any time. The study procedure was approved by the local ethics committee.

4.2.2 Apparatus

Stimulus Intensity Scores. Subjectively perceived stimulus intensity was assessed by an Electronic Visual Analog Scale (EVAS). This customized EVAS device consists of a continuously turning knob, whose activity is displayed on the internal LCD screen (13 cm × 4 cm). Additionally, anchoring adjectives can be presented onto that screen. In this study, the anchors were “very low intensity”, “medium intensity”, and “very high intensity”. Response resolution of the EVAS device is 256-point. Recent studies confirmed the validity of electronic VAS devices in judging the intensity of startle stimuli (Swerdlow et al., 2007). We standardized the judgments by the scale resolution and thus provided %EVAS scores.

Data Recording Parameters. Physiological data were collected via a Biopac® MP150 system (Biopac Systems, Inc.) with 16 bit resolution and a sampling rate of 1 kHz. EMG startle responses were recorded via Tyco Healthcare H124SG electrodes (diameter: 24 mm) placed below the left eye with an inter-electrode distance of 1.5 cm to assess EMG-activity of the

Musculus orbicularis oculi. Hardware band-pass filter settings (Biopac EMG100C) were 10-500 Hz, followed by software filtering (28 Hz high-pass cutoff: (van Boxtel et al., 1998). The raw signal was rectified and integrated online with a time constant of 10 ms (Blumenthal, 1994). Electrodes for ECG measurement (ECG Tyco Healthcare H34SG Ag/AgCl electrodes of 45 mm diameter) were placed according to a standard lead II configuration. The ECG signal was high-pass filtered (Biopac ECG100, HPF: 0.5 Hz) and stored to disk (1 kHz), as well. R-waves were identified online by a fast DASYLAB-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 sinus rhythm was higher than 99.8 %, with a latency below 3 ms (internal lab report).

Stimulus Presentation. Acoustic startle stimuli (intensities: 85, 95, and 105 dB(A), 50 ms duration, instantaneous rise time, binaural presentation) were presented via headphones (Sennheiser Electronic GmbH & Co. KG, Wedemark, Germany). Experimental instructions were displayed by an E-Prime 1.1 (PST Software, Inc.) based platform onto a LCD monitor at a distance of 80 cm from participant to screen.

Response Times Data Collection. A home button and a response button were located on a PST E-Prime Serial Response Box (PST Software, Inc.) with a distance of about 1 cm. While the middle key of the Response Box was always defined as the home button, the response button was located left or right of the home button, each for half of the participants. As in past studies (Doucet & Stelmack, 1999; Doucet & Stelmack, 2001; Jensen & Munro, 1979), the time from onset of stimulus presentation to lifting off the home button was defined as reaction time (‘RT’), representing the central component of the response time. The interval between lifting off the home button and pressing the response button was defined as movement time (‘MT’), representing the motor component of the response time. Median values were calculated for each condition in a particular participant and included in the statistical model due to the non-linearity of response times. Means of medians over participants are reported in the results section.

4.2.3 Analysis of Physiological Data

A customized C++ based semi-automated PC program was used to analyze EMG-responses. The algorithm identified response peaks in the rectified and integrated signal during a time interval of 20 to 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 inspected manually after algorithmic detection. Signals with electrical and physiological artifacts, such as coinciding blinks or other facial muscular activities, which introduced noise to the baseline period and made the correct quantification of the response impossible, were rejected from analysis and defined as missing. If responses were not visible (zero amplitude) at the typical response latency of a particular participant, 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 within-participant, calculated separately for each startle stimulus intensity, and according to whether startle was elicited during the early or late cardiac cycle phase.

4.2.4 Procedure

Participants were seated in a comfortable chair in front of a LCD computer screen. Electrodes for ECG measurement were placed according to a standard lead II configuration. Glasses were removed and EMG electrodes were attached below the left eye to assess activity of the M. orbicularis oculi. Headphones were attached. Participants were then allowed to put their glasses back on and 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. When hearing a noise, participants had to respond as fast as possible by lifting the finger from the home button and pressing the response button. After each stimulus they judged the subjective intensity of the noise on the EVAS device.

At the beginning of the experimental session six startle probes without any contingence to the participants' heartbeat served as habituation trials. EMG-responses on these trials were not further analyzed. During the experimental procedure startle probes were presented with a jittering inter-stimulus-interval of 8-12 seconds, according to a completely randomized

sequence over all experimental conditions: startle stimuli at an intensity of 85, 95, or 105 dB(A) appeared randomly with a latency of 230 ms (early cardiac cycle phase) or 530 ms (late cardiac cycle phase) after the detection of an ECG-recorded R-wave (see previous study protocol of Schulz and colleagues (2009a). In each of these 3 × 2 = 6 conditions, 10 stimuli were presented, 60 stimuli overall. Total length of the experimental session, including instructions, was about 20 minutes.

4.2.5 Statistical Analysis

A 3 × 2 ANOVA with repeated measurement was employed for each dependent variable with variations of the within-subject factors 'startle stimulus intensity' (85, 95, and 105 dB(A)) and 'cardiac cycle phase' (R-wave +230 ms and R +530 ms). Dependent variables were (i) EMG startle response magnitude, (ii) judged stimulus intensity via the EVAS device, (iii) reaction time to the startle stimulus, (iv) movement time, and (v) total response time. Critical α-level was set to .05. For any effect with repeated measurement and more than two conditions, Greenhouse-Geisser corrected *p*-values are reported. For post-hoc analyses, dependent *t*-tests were calculated. All statistics were conducted with SPSS 15.0 (SPSS, Inc.).

4.3 Results

To assure that all R-waves were detected correctly, we inspected the ECG data to determine whether any participant suffered from irregular heartbeats. However, all participants had a regular sinusoidal cardiac rhythm without premature heartbeats and a resting heart rate of lower than 85 bpm.

EMG data of two participants were excluded from further analysis due to the absence of any visible eye blink response. Thus, EMG startle response magnitude data of 23 participants (13 women; mean age: 25.1; SD = 4.2 y; mean HR: 75.2; SD = 7.2 bpm; mean BMI: 21.5; SD = 1.4 kg/m²) remained.

Behavioral data of five participants were incomplete because of a technical malfunction during the second half of the experimental procedure.

4.3.1 EMG Startle Response Magnitude

ANOVA for startle response magnitude revealed significant main effects of startle stimulus intensity (F[2,44] = 28.04; $p < .001$; $\eta^2 = .56$) and cardiac cycle phase (F[1,22] = 6.46; $p < .025$; $\eta^2 = .23$). Startle response magnitude was higher in conditions of more intense stimulation, 105 dB(A) (M = 103 µV; SEM = 18 µV), 95 dB(A) (M = 77 µV; SEM = 14 µV), and 85 dB(A) intensity (M = 50 µV; SEM = 10 µV). All pairwise comparisons of stimulus intensity were significant ($p < .001$). As expected, startle response magnitude was attenuated during the early cardiac cycle phase (R-wave +230 ms: M = 73 µV; SEM = 13 µV), compared to the late cardiac cycle phase (R +530 ms: M = 80 µV; SEM = 15 µV). This result agrees with previously reported effects of cardiac modulation of startle eye blink (Nyklicek et al., 2005; Schulz et al., 2009a; Schulz et al., 2009b). However, a marginally-significant interaction of startle stimulus intensity and cardiac cycle was found (F[2,44] = 2.82; $p = .073$; $\eta^2 = .12$). Post-hoc tests confirmed significance of the cardiac cycle effect only for the startle stimulus intensity of 105 dB(A) ($p < .025$), and marginal significance at 95 dB(A) ($p = .059$), but not at 85 dB(A) ($p > .10$) (see Fig. 4). We conclude that the cardiac modulation effect of startle eye blink depends on the intensity of the startle stimulus.

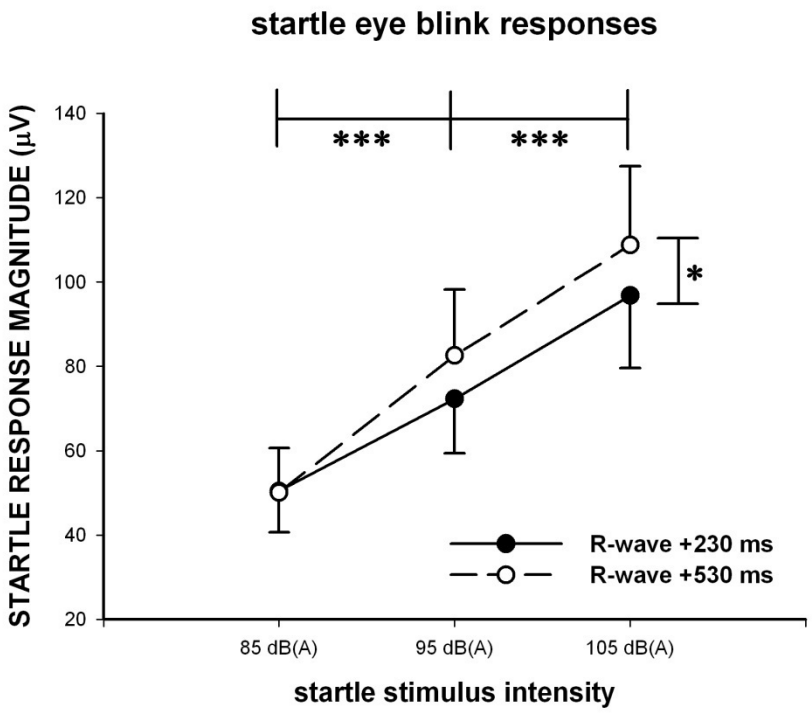

Figure 4. The startle eye blink responses assessed by unilateral EMG. Both main effects of stimulus intensity ($p < .001$) and cardiac cycle phase ($p = .019$) were significant (error bars: SEM).

4.3.2 Subjectively Perceived Intensity

As expected, a strong main effect of stimulus intensity on the subjective intensity ratings was found ($F[2,48] = 404.50$; $p < .001$; $\eta^2 = .94$). Mean EVAS-based intensity judgments (%EVAS) were 23.6 (SEM = 0.86) at 85 dB(A) intensity, 47.5 (SEM = 1.71) at 95 dB(A) intensity, and 73.0 (SEM = 1.88) at 105 dB(A) intensity. All pairwise comparisons of stimulus intensity were significant ($p < .001$). Furthermore, we observed a significant main effect of cardiac cycle ($F[1,24] = 5.56$; $p < .05$; $\eta^2 = .19$), indicating a less intense judgment when startle stimuli were presented at R +230 ms (M = 47.4; SEM = 1.24), compared to R +530 ms (M = 48.7; SEM = 1.18) (see Fig. 5). The significant interaction of stimulus intensity and cardiac cycle ($F[2,48] = 4.02$; $p < .05$; $\eta^2 = .14$) suggested that the impact of the cardiac cycle on subjectively perceived intensity was present at the 105 dB(A) ($p < .01$) and 95 dB(A) ($p < .05$) intensities, but not in the 85 dB(A) intensity condition ($p > .10$).

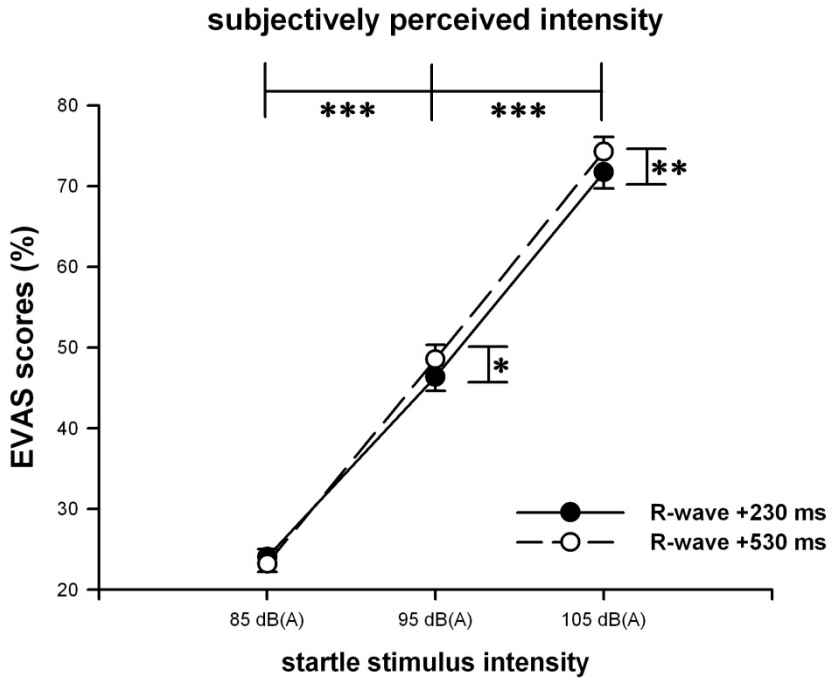

Figure 5. Subjectively reported stimulus intensity scores of the startle noises reflect much the same effects that were observed in startle eye blink responses. The effect of cardiac cycle is seen at 95 and 105 dB(A) intensity.

4.3.3 Response Time Components

Response Time. Replicating recent results, we found a main effect of stimulus intensity on response time (F[2,48] = 12.68; $p < .001$; $\eta^2 = .35$), indicating accelerated total response times (= reaction time + movement time) to higher startle intensities: 105 dB(A) (M = 422 ms; SEM = 24.5 ms); 95 dB(A) (M = 440 ms; SEM = 24.5 ms); 85 dB(A) (M = 465 ms; SEM = 27.2 ms; see Fig. 6a). Post-hoc analysis revealed significant differences in response time between all three intensity conditions (85 vs. 95 dB(A): $p < .025$; 95 vs. 105 dB(A): $p < .05$; 85 vs. 105 dB(A): $p < .001$). Furthermore, neither the main effect of cardiac cycle (F[1,24] < 1) nor the stimulus intensity × cardiac cycle interaction (F[2,48] < 1) reached significance. Response time was not affected by cardiac cycle phases, at any intensity of startle stimuli.

Reaction Time. The ANOVA for the central, evaluative, component 'RT' revealed an even stronger effect in the same direction as was found for the response time. The main effect of stimulus intensity was significant (F[2,48] = 15.81; $p < .001$; $\eta^2 = .40$). Reaction time was shortened at a startle stimulus intensity of 105 dB(A) (M = 273 ms; SEM = 12.1 ms), compared to a 95 dB(A) intensity (M = 283 ms; SEM = 13.5 ms) and an 85 dB(A) intensity

(M = 300 ms; SEM = 15.2 ms), and all pairwise comparisons reached significance (85 vs. 95 dB(A): $p < .01$; 95 vs. 105 dB(A): $p < .025$; 85 vs. 105 dB(A): $p < .001$). In support of our hypothesis, there was a significant main effect of cardiac cycle (see Fig. 6b; $F[1,24] = 4.74$; $p < .05$; $\eta^2 = .17$). RT was prolonged during the early cardiac cycle phase (R +230 ms: M = 289 ms; SEM = 14.2 ms) compared to the late cardiac cycle phase (R +530 ms: M = 281 ms; SEM = 12.9 ms). This effect reached statistical significance in the condition of 85 dB(A) stimulus intensity ($p < .025$), but not in the 95 dB(A) condition ($p > .10$), nor in the 105 dB(A) intensity condition ($p > .10$). However, no significant stimulus intensity × cardiac cycle interaction was found ($F[2,48] = 1.05$; $p > .10$).

Movement Time. The analysis of the motor component 'MT' also showed a main effect for stimulus intensity ($F[2,48] = 4.21$; $p < .025$; $\eta^2 = .15$). Again, higher startle stimulus intensity led to faster movement times (105 dB(A): M = 149 ms; SEM = 17.2 ms; 95 dB(A): M = 158 ms; SEM = 16.2 ms; 85 dB(A): M = 165 ms; SEM = 18.6 ms; see Fig. 6c). However, pairwise significance was only found between the conditions of 85 dB(A) and 105 dB(A) intensity ($p < .01$). This suggests that the accelerating effect of stimulus intensity on response time is more likely caused by the evaluative component ('RT') of the response time than by the motor component ('MT'). The main effect of cardiac cycle on MT also reached statistical significance ($F[1,24] = 7.55$; $p < .025$; $\eta^2 = .24$). MT was shortened during the early cardiac cycle phase (R +230 ms: M = 152 ms; SEM = 17.4 ms) compared to the late cardiac cycle phase (R +530 ms: M = 162 ms; SEM = 16.9 ms). Post-hoc analyses revealed that this effect was present in the conditions of 85 dB(A) ($p < .05$) and 95 dB(A) ($p < .05$) stimulus intensity, but not at 105 dB(A) ($p > .10$) (see Fig. 6c). However, the interaction of stimulus intensity and cardiac cycle was not significant ($F[2,48] < 1$).

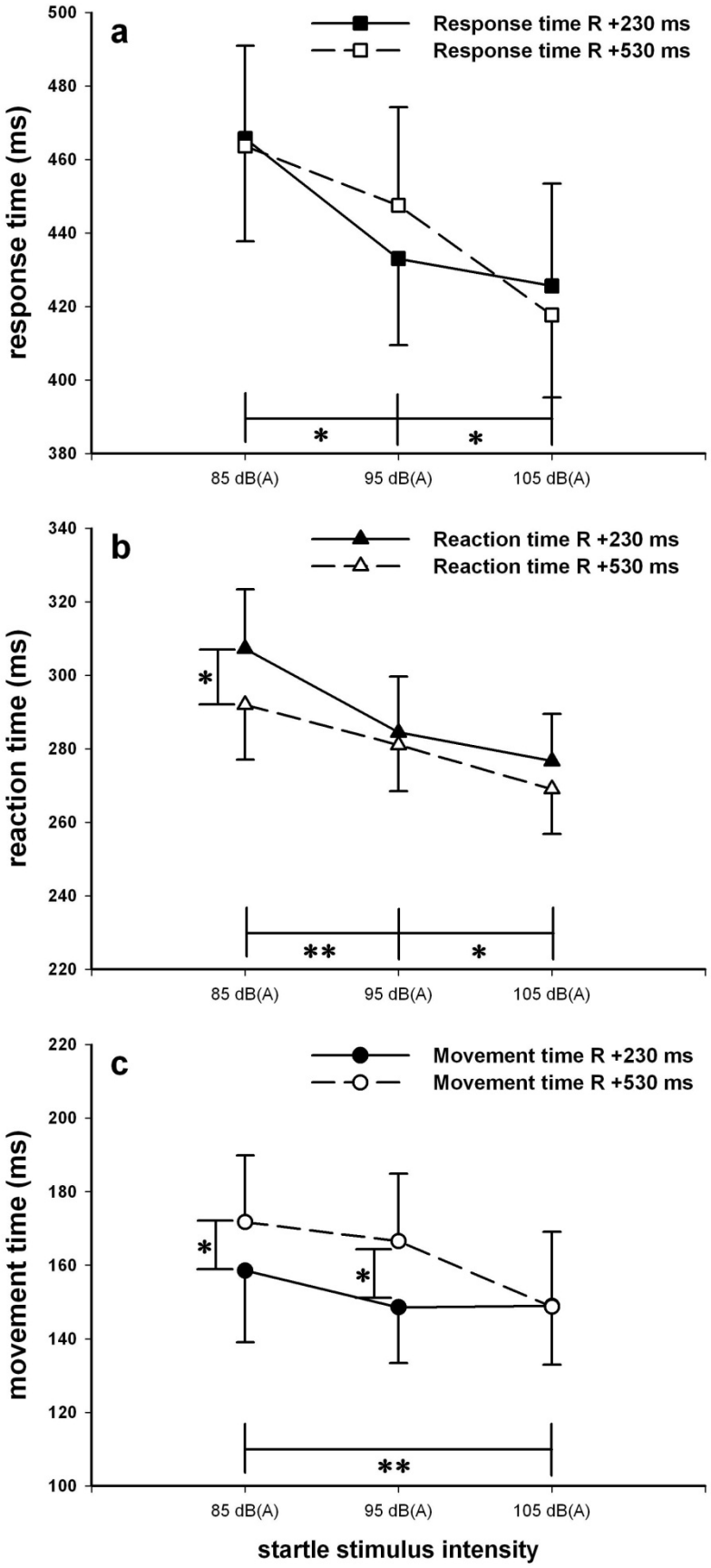

Figure 6. The total response time (a) was divided into the evaluative component (b; reaction time: 'RT') and the motor component (c; movement time: 'MT'). Means of medians are reported.

4.4 Discussion

This study aimed to replicate an inhibitory effect of the early cardiac cycle phase on the startle eye blink (CMS) and to investigate whether such a cardiac modulation effect is also present in other cognitive processing of the incoming startle stimulus. In support of previous research, startle response magnitude was diminished during the early cardiac cycle phase (R-wave +230 ms), compared to the late cardiac cycle phase (R +530 ms). This effect was most prominent at higher stimulus intensities. The principal new finding of this study is that higher cognitive processing of startle stimuli is also impacted by the cardiac cycle phase. First, we found lower subjective intensity scores of startle stimuli when they were elicited during the early cardiac cycle phase. Second, the analysis of psychomotor responses to the startle stimuli revealed a prolongation of the evaluative component, while the motor component (movement time) was shortened, such that the total response time remained unaffected during the early cardiac cycle.

As expected, the intensity of the startle stimuli had a strong facilitative effect on the eye blink response, as was shown in earlier studies (Blumenthal, 1988; Blumenthal & Goode, 1991; Yamada, 1983). Higher startle stimulus intensities also resulted in substantially higher subjective intensity ratings. Furthermore, the evaluative component, the motor component, and the total response time were shortened at higher startle stimulus intensities. Thus, both the stimulus evaluation, as was previously reported (Carlsen et al., 2007), and the execution of a motor response, are facilitated at higher startle stimulus intensities.

Earlier research has shown that responses to various reflex-eliciting stimuli are dampened during the early phase of the cardiac cycle (Edwards et al., 2002; Edwards et al., 2001; Nyklicek et al., 2005; Schulz et al., 2009a; Schulz et al., 2009b). Since this effect relies on intact baro-afferent signal transmission, the short-term loading and unloading of arterial baroreceptors are probably responsible for the effect (Schulz et al., 2009a; Schulz et al., 2009b). This assumption is supported by many findings that suggest a maximal baro-afferent neural transmission, originated by the arterial pulse wave, at a latency of 200 to 250 ms after the actual R-wave (Donadio, Kallio, Karlsson, Nordin & Wallin, 2002; Eder, Elam & Wallin,

2009; Edwards et al., 2003; Edwards et al., 2009). A long-term increase of baroreceptor loading, which is observed in individuals with arterial hypertension, affects the cognitive processing of aversive stimuli, as in the increase of subjective pain thresholds (Sheps et al., 1992; Zamir & Shuber, 1980). Furthermore, several studies have found impaired performance on simple cognitive tests in hypertensives, such as simple reaction time tasks (Blumenthal et al., 1993; Harrington et al., 2000; Karla et al., 1993). While this impairment in reaction time tasks was also found during the early cardiac cycle phase (Edwards et al., 2007; Stewart et al., 2006; Weisz & Adam, 1996), these findings have not been accompanied by a modulated subjective intensity rating of the delivered stimuli (Edwards et al., 2003). In contrast to those earlier findings, our study showed that a lower startle eye blink response during the early cardiac cycle phase corresponded with lower intensity ratings of these stimuli. This effect of the cardiac cycle on the subjective intensity of startle stimuli was most prominent in higher intensity conditions. Thus, the cardiac modulation of subjectively perceived intensity may be specifically observed with high intensity stimulation.

The present findings show that at the lowest stimulus intensity, reaction time is prolonged in the early cardiac phase, relative to the late phase. However, at the same intensity condition, movement time is faster in the early than the late cardiac phase. Although the pre-attentive startle reflex and higher cortical processing of the reflex-eliciting stimuli are both modulated by the cardiac cycle phase, it is difficult to link processes like the judging of intensities or the psychomotor responding to distinct brain areas (Swerdlow et al., 2005). The inhibition of pre-attentive and attentive operations during the early cardiac cycle phase may, at first glance, suggest a general attenuation of sensory input. However, two findings argue against this: First, a previously found lateralization effect that reveals a specificity of the cardiac modulation to left-ear stimulation (Schulz et al., 2009b) makes an inhibition of sensory input implausible, since a lateralization effect implies a hemispheric-specificity of neural pathways. Second, the present study found discrepant effects of the cardiac cycle phase on the evaluative and motor components of the response time, although both components show similar changes with altered stimulus intensity. With this study we cannot explain the discrepant stimulus intensity findings, indicating cardiac cycle time effects on perceived intensity for *higher* intensities, whereas cardiac cycle time effects on psychomotor reactions seems to be more pronounced at *lower* intensities. However, the latter interactions (for RT and MT) were not

statistically significant. This aspect deserves further exploration in future studies with higher statistical power.

These converse effects of the cardiac cycle on stimulus evaluation and motor response times may originate from an evolutionary-adaptative function: A phasic increase of baroreceptor loading is usually caused by an elevation of blood pressure. This appears, for instance, in a potentially dangerous context to provide a higher level of energy to the organism for the preparation of a motor response (Kopin, 1995). Under this condition, the organism may benefit more from the execution of motor evasive responses (fight-or-flight), but less so from the detailed cognitive evaluation of incoming environmental stimuli. This assumption matches with the finding that the selectivity of attention is improved in stress (Chajut & Algom, 2003). A second advantage may be related to a specific adversity-reducing effect of baro-afferent stimulation, which is known to contribute to stress-induced hypoalgesia (Dworkin et al., 1979; France, 1999). Nevertheless, the postulation of any adaptive benefit remains highly speculative, and the current experimental context did not represent an ecologically valid stressful situation. A possible future approach to discover the effects of baro-afferent feedback on selective cognitive functions may involve focusing on attentional networks that are assumed to be differently affected by activation and arousal (Wang & Fan, 2007). An alternative explanation is that stimulus intensity perception may require more cognitive resources than the mere initiation of a motor reaction in response to the simple appearance of a go-stimulus, and thus may be easier in conflict and resource-competition with ascending visceral neuro-traffic. However, the startle methodology used here may be optimal to identify the evaluational and motor capacities separately across the cardiac cycle for two reasons: First, because both components reflect complex processes, which are difficult to decompose, except for very rapid responses with a natural analog of stimulus parameter (i.e. intensity) and response speed. Second, baroreceptor stimulation and appearance of a potentially harmful stimulus have to occur within a very short and exact timeframe. This may be realized most accurately with the short latencies between stimulus presentation and processing of startle reflex elicitation (Koch, 1999).

Two brainstem-relayed reflexes are involved in the cardiac modulation of startle eye blink (Schulz et al., 2009a): first, the primary acoustic startle circuit (Cochlear root neurons,

Nucleus reticularis pontis caudalis, neurons in the Facial nucleus) (Davis et al., 1999), and second, the baroreflex circuit (Nucleus tractus solitarius, Nucleus ambiguus, Cardiac pacemaker) (Jänig, 2006). The present study demonstrates that the influence of the cardiac cycle on startle eye blink is also reflected by effects on subjective intensity ratings and response times, which both clearly require higher cognitive, attentional processes. Cortical structures that process sensations from the cardiovascular systems (e.g., right Anterior insula, Anterior cingulum) (Critchley et al., 2004; Pollatos et al., 2005) may also play a role in the cortical relaying of cardiac modulated stimuli. As an alternative to other paradigms for assessing sensations from the cardiovascular system, such as heartbeat counting tasks (Schandry, 1981a) and interoceptive-exteroceptive discrimination tasks (Whitehead et al., 1977), the cardiac modulation of startle paradigm does not require participants' active cooperation. Previous to the present study, it has been unclear whether the validity of both types of paradigms is comparable, since it was not yet proven that the cardiac modulation of startle is also reflected by higher cognitive processing. The present study answers this question.

In previous literature, different paradigms are described to distinguish the evaluative and the motor components of response time. The authors of some studies have decomposed the total response time by measuring the lift-off of a home button and the pressing on a response button relative to the stimulus onset (Doucet & Stelmack, 1999; Doucet & Stelmack, 2001; Jensen & Munro, 1979), while others focus on the onset of EMG assessed activity of a relevant muscle (Carlsen et al., 2007; Edwards et al., 2007). Since these methodologies are not fully comparable, the results may not be either. However, in both methods, the length of a cognitive process (i.e. the response time) is defined via the onset of a motor process. Although these methods may validly reflect the underlying process, it should be considered whether electroencephalographic methods (Hackley & Valle-Inclan, 1998) may assess the central component of the response time more precisely. Future studies should address this issue.

4.4.1 Limitations

The presence of the effect of the cardiac cycle phase on startle eye blink, subjective intensity, and response time components is, in this study, dependent on parameters of the eliciting stimulus. However, due to the moderate sample size the study may be underpowered, and not

suitable for an interpretation of some non- and marginally-significant interaction effects, since the effect of the cardiac cycle under lower intensity conditions may be small. A more extensive exploration of required stimulus parameters to observe the effect in the future may allow more detailed hypotheses about involved brain structures. An extension of the present study protocol to a wider range of stimulus intensities, for instance, would also prevent possible anchoring effects in the judging of subjective intensity that may cover less intense modulation effects.

Although the modulatory effects of the cardiac cycle on the startle response was already shown over more latency conditions after the R-wave (0, 100, 230, 530 ms) in an earlier study (Schulz et al., 2009b), the key effects of the present study were only studied at one early and one late latency. Compared with those earlier works, we used fewer stimuli repetitions and a smaller sample size, which was additionally reduced by two non-responders. That may influence the comparability of these and earlier results, although the fundamental effect of startle inhibition at the earlier R-interval was replicated. Thus, a more detailed exploration of the modulation effect across the whole cardiac cycle may be suggestive.

Finally, we investigated only healthy individuals in this study. However, a responsibility of arterial baroreceptors for the effects found would be emphasized if patients with impaired autonomic functions did not show the described response pattern. Although former studies indicate a key role of baroreceptors for the cardiac modulation effect on startle (Nyklicek et al., 2005; Schulz et al., 2009a), the involvement of other processes in the described effects, such as an effect of prepulse inhibition (PPI) of heartbeats on eye blink, subjective intensity, and response times, cannot be excluded.

4.4.2 Conclusion

The cardiac cycle was repeatedly shown to modulate startle responses to acoustic stimuli. This modulating effect (CMS) was also reflected by lower subjectively perceived intensities of startle stimuli that were elicited during the early cardiac cycle phase, and modulating effects on the evaluative and motor component of the response time to those stimuli. These

findings suggest that the rapid and pre-attentive modulation of startle responses via brainstem-located neural structures may also impact on higher cognitive, attentive processes.

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4.ii AUTHOR NOTES

For reprint requests, please correspond to: Dipl.-Psych. André Schulz, University of Trier, Institute of Psychobiology, Johanniterufer 15, D-54290 Trier, E-Mail: andre.schulz@uni-trier.de.

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Chapter V:

Cardiopulmonary Baroreceptors Affect Reflexive Startle Eye Blink

(Richter, Schulz, Port, Blumenthal & Schachinger, 2009)

First Author: Steffen Richter.

Co-Authors: Johannes Port, Terry D. Blumenthal & Hartmut Schächinger.

5.0 Abstract

Baro-afferent signals originating from the 'high pressure' arterial vascular system are known to impact reflexive startle eye blink responding. However, it is not known whether baro-afferent feedback of the 'low pressure' cardiopulmonary system loading status exerts a similar effect. Lower Body Negative Pressure (LBNP) at gradients of 0, -10, -20, and -30 mmHg was applied to unload cardiopulmonary baroreceptors. Acoustic startle noise bursts were delivered 230 and 530 ms after spontaneous R-waves, when arterial baroreceptors are either loaded or unloaded. Eye blink responses were measured by EMG, and psychomotor reaction time by button pushes to startle stimuli. The new finding of this study was that unloading of cardiopulmonary baroreceptors increases startle eye blink responsiveness. Furthermore, we replicated the effect of relative loading/unloading of arterial baroreceptors on startle eye blink responsiveness. Effects of either arterial or cardiopulmonary baroreceptor manipulations were not present for psychomotor reaction times. These results demonstrate that the loading status of cardiopulmonary baroreceptors has an impact on brainstem-based CNS processes.

***Keywords*:** Acoustic Startle Reflex; Arterial Baroreceptors; Cardiopulmonary Baroreceptors; Lower Body Negative Pressure; Reaction Time

5.1 Introduction

There is growing evidence that neural visceral afferent signals have an impact on higher central nervous system (CNS) processes, such as emotion and cognition (Damasio, 2003; Wiens, 2005). Many of the afferent signals, which ascend via vagal, glossopharyngeal, and

thoracic afferent nerve fibres, originate from phasically active thoracic or abdominal structures, such as the lungs, gastrointestinal organs, and the central cardiovascular system (Critchley et al., 2004). Visceral afferents play an important role in adaptation and homeostasis, and neural baroreceptor feedback is required for controlling heart rate, blood pressure, cardiac workload, and vascular resistance, but has also been found to affect central nervous system (CNS) functions which are not directly linked to cardiovascular regulation, such as pain processing (Dworkin, 2000). Baroreceptors are located in arterial vessel walls of the aortic arch and the carotid sinus (the high pressure system), but also in pulmonary vessels and atria (the cardiopulmonary low pressure system). Due to their distribution and due to the blood vessel wall characteristics, arterial baroreceptors respond to changes in arterial pressure, whereas cardiopulmonary baroreceptors respond to changes in central venous pressure which, during healthy conditions, is proportional to changes in central venous volume. Loading of baroreceptors enhances their neural output, unloading induces the opposite effect.

The CNS structures prominently involved in both baroreflex pathways are located in the brainstem. Information from arterial and cardiopulmonary baroreceptors converges in the Nucleus tractus solitarius (NTS), and is further relayed to the Nucleus ambiguus and the Ventrolateral medulla (Aicher et al., 1995; Jänig, 2006; Jeske et al., 1995; Masuda, Terui, Koshiya & Kumada, 1991). Both baroreceptor systems project, via the NTS, to similar CNS structures (e.g., Anterior cingulum, Insular cortex, Locus coeruleus) which have been shown to be involved in pain processing, emotion, and regulation of higher cognitive-motor functions (Grindstaff, Grindstaff, Sullivan & Cunningham, 2000; Henderson et al., 2004; Kimmerly et al., 2005).

Several psychophysiological effects of loading or unloading arterial baroreceptors have been described. Enhanced arterial baroreflex afferent feedback activity impacts on EEG activity (Mini, Rau, Montoya, Palomba & Birbaumer, 1995; Vaitl, Gruppe, Stark & Possel, 1996), attenuates pain perception (Edwards, Inui, Ring, Wang & Kakigi, 2008; Edwards et al., 2003; Edwards et al., 2002; Edwards et al., 2001; McIntyre, Edwards, Ring, Parvin & Carroll, 2006; Mini et al., 1995), and induces a prolongation of psychomotor reaction times (Edwards et al., 2007; McIntyre, Ring, Edwards & Carroll, 2008b; Vaitl et al., 1996), and unloading vs. loading of arterial baroreceptors may affect memory processes (Moor et al., 2005).

Furthermore, neural arterial baroreceptor afferent feedback transmission has an inhibitory effect on simple brainstem reflexes, such as the startle response (Nyklicek et al., 2005; Rau et al., 1993; Schulz et al., 2009a; Schulz et al., 2009b). The startle eye blink reflex is a protective reflex which is reliably evoked by presentation of abrupt and intense acoustic noise stimuli. This reflex is affected by spontaneous neural arterial baroreceptor afferent feedback transmission, since lower startle responsiveness was found when stimuli were presented during the early cardiac cycle phase, when arterial baroreceptors are loaded, as compared to the late cardiac cycle phase, when arterial baroreceptors are relatively unloaded. This effect relies on intact neural afferent signal transmission, and it is absent in diabetic autonomic neuropathy (Schulz et al., 2009a).

However, in contrast to arterial baroreceptors, relatively little is known about the significance of cardiopulmonary baroreceptors for psychophysiological processes. The loading status of cardiopulmonary baroreceptors may change with everyday activities, such as altering body position from laying/supine to standing upright (Pump et al., 1997; Pump et al., 2001a; Pump, Kamo, Gabrielsen & Norsk, 2001b). It may be reduced during moderate states of dehydration (i.e. due to reduced drinking or increased water loss), as well as substantial blood loss (Abboud, Eckberg, Johannsen & Mark, 1979; Wada et al., 1995). Furthermore, emotional stress may increase central venous volume and pressure (Brod et al., 1979), as well as pulmonary artery pressure (Schachinger, Grob, Ritz & Soler, 2000), and thus affect the loading status of cardiopulmonary baroreceptors. An impact of cardiopulmonary baroreceptor activity on pain processing (D'Antono, Ditto, Sita & Miller, 2000; Randich & Maixner, 1984; Sheps et al., 1992; Vaitl et al., 1996) and EEG theta-band activity (Vaitl & Gruppe, 1990) has been reported, and the direction of effects is similar to those of arterial baroreceptor manipulations. Since relative arterial baroreceptor unloading is associated with increased startle eye blink responsiveness, it may be assumed that cardiopulmonary baroreceptor unloading induces a similar effect, but this has never been investigated. The current research project was designed to replicate the finding that relative unloading of arterial baroreceptor enhances startle responsiveness, and, at the same time, to investigate whether unloading of cardiopulmonary baroreceptors affects startle responsiveness in the same direction. Statistical interaction effects of arterial and cardiopulmonary baroreceptors on startle eye blink responsiveness will also be tested, as well as baroreceptor loading and unloading effects on a psychomotor reaction time task (button press) to startling stimuli.

This study employed Lower Body Negative Pressure (LBNP), which induces central vascular volume changes with only minor impact on mean arterial blood pressure (Franke, Johnson, Steinkamp, Wang & Halliwill, 2003; Hinghofer-Szalkay et al., 1996; Kitano et al., 2005; Laszlo et al., 1998; van Hoeyweghen et al., 2001), and thus may be considered as being 'non-hypotensive'. One of the greatest advantages of LBNP is related to its dose-response characteristics; that is, increasing LBNP stepwise increases its effects (Convertino, Cooke & Holcomb, 2006; van Hoeyweghen et al., 2001). Furthermore, it can be assumed that LBNP has very little body position-related effect on somatic muscle tone and stimulation-associated perceptions, since body and limb position remain largely unchanged during LBNP. Thus, no change in proprioceptive sensations is expected. Also, LBNP is not painful and it does not trigger aversive sensations. However, as there are no studies focussing on potential mood changes during LBNP, and since mood changes may have an impact on startle, this study will assess affective changes in order to control for this.

To clarify the roles of arterial and cardiopulmonary baroreceptors in modulating startle processing we conducted a within-participant experiment with 12 healthy men. The participants received 240 acoustic startle noises in total, presented via headphones, with latencies of either 230 ms (arterial baroreceptor loading) or 530 ms (arterial baroreceptor unloading) after the cardiac R-wave, while each participant was treated with a series of 5 minutes of LBNP at levels of 0, -10, -20, and -30 mmHg each, in randomized order. For each participant, every LBNP level was applied two times. Surface electromyogram (EMG) of the orbicularis oculi muscle was recorded and the participants were asked to respond to each noise as quickly as possible by pressing a response key. The latter was done because several studies have shown that arterial baroreceptors affect psychomotor reactions (Edwards et al., 2007; McIntyre et al., 2008b; Stewart et al., 2006; Weisz & Adam, 1996), but little is known about the effect of cardiopulmonary baroreceptors on psychomotor function. In general, we expected to find an inhibitory effect of both baroreceptor types on startle responsiveness and psychomotor reactions.

5.2 Methods

5.2.1 Participants

We studied 12 healthy male non-smokers who participated voluntarily and received a small monetary incentive of € 25,-. Mean age was 25 years (range: 20 to 34 years; SD = 4.0), mean resting heart period was 1048 ms (SD = 170), and mean BMI was 24 kg/m² (SD = 2.3). All participants had a regular sinus rhythm. Exclusion criteria were BMI below 18 or above 30 kg/m², and a height of more than 1.95 m (because of the LBNP-chamber size). Participants with any chronic or acute diseases, cardiovascular disorders, or known hereditary vascular diseases (e.g. aneurysms) in their family history were excluded from the study. Women were not included so as to increase homogeneity of our sample. Participants underwent a routine medical history and screening that included a standard electrocardiogram (ECG) recording, resting blood pressure measurement, and blood analysis (standard haematology and chemistry). None of the participants showed any pathological findings and total serum cholesterol levels were lower than 180 mg/dl for each participant. All participants gave written informed consent. The study was approved by the community-based ethical committee of the 'Landesärztekammer Rheinland-Pfalz, Mainz, Germany'.

5.2.2 Apparatus

The LBNP-device consists of a steel 120 × 60 × 50 (L × W × H) cm chamber (Curio Medizinelektronik, Bonn, Germany) fixed on a table that accommodates the participant's legs. An adjustable bicycle seat is placed in the chamber to ensure a stable position of the participant. The chamber is sealed with a neoprene cover at the level of the iliac crest. An adjustable acrylic glass cover avoids deformation of the neoprene during negative pressure gradients. Attached above the participant's head is a LCD monitor for written instructions during the experiment. The negative pressure within the chamber is electronically controlled and infinitely variable from -10 mmHg to -60 mmHg. Any selected negative pressure within the chamber was reached in less than 5 seconds. The vacuum pump was positioned in a soundproof box at a distance of 1 m from the participant. Ambient noise level in the laboratory was at 44 dB(A), the maximum ambient noise level with the vacuum pump at maximum performance level was 47 dB(A).

5.2.3 Stimulus Control

All stimuli and written instructions were delivered by E-Prime 1.2 software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). Startle stimuli consisted of 50 ms white noise at 105 dB(A) with instantaneous rise time. The headphones for binaural stimulation (Sennheiser electronic GmbH & Co KG, Wedemark, Germany) covered the entire ear and reduced ambient noise markedly (passive noise reduction > 10 – 40 dB(A), according to Sennheiser product information). No participant was able to correctly report acoustic activity of the vacuum pump when headphones where attached. The experimenter sat in the same room for safety reasons, but out of sight of the participant. Movement sounds or other indicators of the experimenter's presence could not be heard by the participant due to the sound-reducing properties of the headphones.

5.2.4 Ratings

Participants rated affective valence, momentary arousal, and perceived intensity of ambient noise at every LBNP level. The affective rating scale was a paper-pencil based single item 20 cm visual analog rating scale. Questions were presented on separate pages to avoid carry-over effects. Anchors were "very unpleasant" on the left and "very pleasant" on the right for affective valence, "very low" and "very high" for arousal, and "none" and "very high" for ambient noise intensity. Ratings were measured in mm and transferred into a range of 0 to 100.

5.2.5 Recording Parameters

Standard Ag/AgCl electrodes (ECG Tyco Healthcare H34SG Ag/AgCl electrodes of 45 mm diameter) were used for ECG and impedance cardiography (ICG, see below). ECG electrodes were attached to obtain a standard lead II configuration. ICG spot-electrodes were attached on both sides of the neck, below each Clavicula, on both sides of the Arcus costae and on the Lower abdomen in the Mid-clavicular line. ECG and EMG were recorded with 1000 Hz sampling rate at 16 bit resolution by a Biopac MP150 system, with amplifier modules ECG100C and EMG100C. Hardware band-pass filter settings were 0.5 to 500 Hz for ECG and 10 to 500 Hz for EMG. The R-waves were identified by a customized ECG detection device (Curio Medizinelektronik, Bonn, Germany). Accuracy of R-wave detection in sine-

rhythm was higher than 99.8%, with latency below 3 ms (internal lab report). ICG was recorded with 1000 Hz sampling rate at 14 bit resolution by a newly developed research impedance module manufactured at the Institute of Medical Technology (Prof. J. Nagel, Dr. J. Port), University of Stuttgart, Germany. Systolic, diastolic, and mean blood pressure was measured with a Dinamap monitor (Dinamap SX 1846, Critikon, USA). A handheld reaction time-button was placed into the participant's dominant hand, and responses were recorded by E-Prime software (see above). Data were stored to hard disk with Labview-based software (National Instruments, USA).

5.2.6 Psychophysiological Data Analysis

The EMG signal was further filtered in software with a passband of 28 to 500 Hz (van Boxtel et al., 1998), and then integrated offline with a 5 ms Boxcar filter (the equivalent of a 10 ms time constant) (Blumenthal, 1994; Blumenthal et al., 2005) with AcqKnowledge software (Biopac Systems Inc., Goleta, CA, USA). It was then processed offline with a proprietary C++ based semi-automated PC program running on a WinXP platform. The algorithm identified response peaks in the rectified and integrated signal in the time interval of 20 to 150 ms after the startle probe onset. The baseline period was defined as the 50 ms interval prior to the acoustic stimulation. All response data were manually confirmed. Signals with electrical and physiological artifacts, such as coinciding eye blinks or other facial muscular activity, were rejected from analysis and defined as missing. If responses were not visible in the typical response latency range of a particular participant, response magnitude 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 participant and according to whether startle was elicited in the cardiac systole or diastole, in each LBNP condition.

Cardiovascular data were processed with WinCPRS software (WinCPRS 1.6, Absolute Aliens Oy, Turku, Finland). Interbeat interval, T-wave amplitude, and heart rate variability were calculated from the ECG. The spectral analysis of interbeat intervals was done with WinCPRS software using a FFT routine. The R-R interval time series was linearly interpolated and resampled with sampling rate of 5 Hz, the resampled data was tapered using a Hanning window and the windowed data zero padded to the next power of 2. The FFT

spectrum was smoothed using a sliding triangular weighting function in order to increase the number of freedoms and thus improve the statistical relevance of the spectrum.

The low frequency band (LF) was defined as 0.06 to 0.14 Hz and the high frequency band (HF) was defined as 0.2 to 0.5 Hz. Normally, the low frequency cut-off of the high frequency band is 0.15 Hz. However, we here used a higher cut-off frequency, so as to exclude the frequency at which startle stimuli were presented (0.14 to 0.2 Hz; corresponding to an ISI of 5 to 7 s). The normalized power of the low frequency band as well as the LF/HF ratio (Task Force, 1996; Berntson et al., 1997; Malliani, Pagani & Lombardi, 1994; Montano et al., 1994; Zaza & Lombardi, 2001) were calculated, because they reflect sympathetic activity more accurately than the absolute power of the low frequency band of heart rate variability, which is also influenced by parasympathetic tone (Akselrod et al., 1985; Pomeranz et al., 1985). The pre-ejection period and left ventricular ejection time were calculated from the ECG and dZ/dt signal. The pre-ejection period was calculated as the interval from R-wave to B-point (corresponding to the opening of the aortic valve), and left ventricular ejection time was calculated as the interval from B-point to X-point (corresponding to the closure of the aortic valve) in the dZ/dt signal. Stroke volume was calculated from the impedance signal by the Kubicek formula (Kubicek, Karnegis, Patterson, Witsoe & Mattson, 1966). Respiratory frequency was calculated with WinCPRS software from changes in the thorax impedance signal (Ernst, Litvack, Lozano, Cacioppo & Berntson, 1999).

5.2.7 Experimental Procedure

The participants visited the lab on a separate day for the medical screening and to familiarize themselves with the experimental setting. On the experimental day, the participants were helped into the LBNP-device, electrodes for ECG, ICG, and EMG were attached, and the Dinamap cuff was fastened to the non-dominant arm. The participants were instructed to relax, avoid unnecessary movement, and press the response button as quickly as possible whenever they perceived a noise stimulus. They received instructions by written information presented on the overhead monitor.

At the beginning of the experimental session, six startle probes were administered as habituation trials, and were not further analyzed. Each participant was then exposed to eight LBNP blocks of either 0, -10, -20, or -30 mmHg pressure gradients, with each LBNP level applied twice to each participant. Dependent variables were averaged across the two instances of each LBNP level. Exposure to LBNP blocks followed a pseudo-randomized order. Each block lasted 5 minutes. After each block was a break of 3 minutes. The first minute of each block was used for technical adjustments and blood pressure measurement. The startle protocol was started after the first minute and lasted between 3 and 3.5 minutes. The difference in startle protocol duration was due to the cardiac cycle dependent elicitation and randomization of startle stimulus presentation. Blood pressure was measured again during the last minute of exposure to LBNP. Intermittent cuff blood pressure readings were taken before and after the startle protocol to avoid interference.

A startle protocol consisted of 30 startle probes that were administered with a randomized interstimulus interval of 5 to 7 seconds. Half of these startle probes were presented 230 ms after an R-wave (cardiac systole), and the other half were presented 530 ms after an R-wave (cardiac diastole), in randomized order. The timing for stimulus presentation in cardiac systole and diastole was chosen according to previous publications (Edwards et al., 2001; Edwards et al., 2007; Ring & Brener, 1992; Ring et al., 1994; Schulz et al., 2009a; Schulz et al., 2009b).

5.2.8 Statistical Analysis

Startle EMG responses were t-transformed to control for between participant differences in startle eye blink responsiveness. For startle data, a 4 × 2 ANOVA with repeated measures design was employed with the factors (i) LBNP level and (ii) cardiac cycle phase. For reaction time data, a similar procedure was used, but the covariate “mean individual reaction time” (the mean of all reaction time data available for that individual) was introduced to control for between participant differences in psychomotor reaction times. For cardiovascular data, an ANOVA with repeated measures design with one factor (LBNP level) was employed, with the respective cardiovascular parameters as a dependent variable. Subjective ratings were transferred from the visual analog scale into a scale from 0 to 100 arbitrary units (AU) and

tested with an ANOVA with repeated measures design with one factor (LBNP level) and the respective rating as the dependent variable.

All *p*-values of within-participants-factors with more than two conditions were adjusted with Greenhouse-Geisser correction, but non-adjusted degrees of freedom are reported in text. A result of $p \leq 0.05$ was considered significant. Statistical analysis was conducted with SAS software (SAS Institute Inc., Cary, NC, USA).

5.3 Results

5.3.1 Cardiovascular and Respiratory Data

Impedance cardiography derived stroke volume could not be calculated for one participant, because B-points were not identifiable in the dZ/dt signal. Systolic blood pressure ($F[3,33] = 4.79$, $p = 0.02$, $\eta^2 = 0.30$) and interbeat interval ($F[3,33] = 13.14$, $p = 0.002$, $\eta^2 = 0.54$) decreased with increasing LBNP. However, mean blood pressure ($F[3,33] = 0.16$, $p = 0.82$) remained unchanged, underlining the non-hypotensive character of the intervention. Diastolic blood pressure increased only slightly ($F[3,33] = 2.64$, $p = 0.081$, $\eta^2 = 0.19$), due to the increase of peripheral resistance induced by LBNP. Thorax impedance was increased by increasing levels of LBNP ($F[3,33] = 52.60$, $p < 0.001$, $\eta^2 = 0.83$) reflecting a reduction of conductive fluids (i.e. blood) in the central compartment, while stroke volume was reduced ($F[3,30] = 25.62$, $p < 0.001$, $\eta^2 = 0.72$), reflecting reduced diastolic filling of the heart.

The pre-ejection period increased (10.4 ms difference for -30 mmHg LBNP on average), and the left ventricular ejection time decreased during higher LBNP (25.6 ms difference for -30 mmHg LBNP on average). Thus, while at -30 mmHg LBNP the interbeat interval decreased by 133 ms on average, the electro-mechanical systole decreased by only 15 ms on average. A summary of systolic time intervals at different levels of LBNP is given in Table 1. The T-wave amplitude decreased with increasing LBNP ($F[3,33] = 21.63$, $p < 0.001$, $\eta^2 = 0.66$). Absolute power in the low and high frequency band of the heart rate variability decreased with increasing LBNP gradients; however, this decrease was only significant for the high frequency band ($F[3,33] = 5.48$, $p = 0.03$, $\eta^2 = 0.33$), indicating parasympathetic withdrawal.

Total power of the heart rate variability also decreased slightly, but not significantly. Normalized high frequency power of the heart rate variability decreased (F[3,33] = 9.95, p = 0.002, η^2 = 0.48), while the low frequency power (F[3,33] = 9.92, p = 0.002, η^2 = 0.47) and the LF/HF ratio increased (F[3,33] = 9.19, p = 0.001, η^2 = 0.46) with increasing LBNP, suggesting decreasing parasympathetic and increasing sympathetic activity. The respiratory frequency decreased with increasing LBNP from 0.31 Hz (SEM = 0.014) at 0 mmHg to 0.28 Hz (SEM = 0.015) at -30 mmHg (F[3,33] = 10.26, p = 0.001, η^2 = 0.48). Mean values and standard errors for cardiovascular data for the different LBNP levels are summarized in Table 1.

Table 1. Summary of cardiovascular parameters observed at 0, -10, -20, and -30 mmHg of LBNP.

Parameter	LBNP 0 mmHg		LBNP -10 mmHg		LBNP -20 mmHg		LBNP -30 mmHg	
	mean	(SE)	mean	(SE)	mean	(SE)	mean	(SE)
systolic blood pressure [mmHg]	112	(2.0)	112	(1.9)	110	(1.4)	109 *	(1.6)
mean blood pressure [mmHg]	84	(1.8)	84	(1.9)	84	(2.3)	83	(1.9)
diastolic blood pressure [mmHg]	65	(1.9)	67	(2.3)	67	(2.2)	68 *	(2.2)
interbeat interval [ms]	1055	(44.0)	1032 *	(40.4)	984 *	(34.1)	922 *	(29.4)
t wave amplitude [mV]	0.30	(0.03)	0.29 *	(0.02)	0.28 *	(0.02)	0.26 *	(0.03)
total power [ms²]	5743	(1418)	5388	(1332)	4768	(1263)	4223	(847)
high frequency [ms²]	956	(290)	816	(262)	531	(118)	254 *	(45)
low frequency [ms²]	1910	(668)	1420	(334)	1158	(332)	1144	(214)
normalized high frequency [NU]	0.49	(0.04)	0.48	(0.05)	0.45	(0.04)	0.33 *	(0.03)
normalized low frequency [NU]	0.50	(0.04)	0.51	(0.05)	0.53	(0.04)	0.67 *	(0.03)
LF/HF ratio	1.18	(0.19)	1.36	(0.27)	1.33	(0.19)	2.10 *	(0.26)
thorax impedance [Ω]	12.6	(0.46)	12.9 *	(0.44)	13.2 *	(0.44)	13.5 *	(0.42)
stroke volume [ml]	79	(8.3)	72 *	(9.9)	58 *	(9.7)	55 *	(9.6)
pre-ejection period [ms]	78.3	(4.3)	80.6	(5.0)	84.6 *	(4.9)	88.7 *	(5.7)
left ventricular ejection time [ms]	334.9	(11.4)	330.7	(14.7)	322.2 *	(14.7)	309.2 *	(14.8)
electromechanical systole [ms]	413.2	(12.4)	411.3	(14.6)	406.8	(15.3)	397.9 *	(15.3)
respiratory frequency [Hz]	0.31	(0.014)	0.30	(0.013)	0.28 *	(0.014)	0.28 *	(0.015)

* Significant differences relative to LBNP of 0 mmHg (based on the within-participant contrasts of the ANOVA with repeated measures design).

5.3.2 Ratings

Statistical test revealed no significant effect of LBNP on affective valence or perceived intensity of ambient noise. Valence ratings were 63.5 (SEM = 3.7) for 0 mmHg, 62.6 (SEM = 3.2) for -10 mmHg, 63.0 (SEM = 3.6) for -20 mmHg, and 62.0 (SEM = 3.2) for -30 mmHg. Given the scale range of 0 to 100, this represents a neutral-to-pleasant state. Ambient noise ratings were 7.6 (SEM = 2.4) for 0 mmHg, 7.5 (SEM = 2.0) for -10 mmHg, 7.8 (SEM= 2.1)

for -20 mmHg, and 8.1 (SEM = 2.1) for -30 mmHg. Given the scale range of 0 to 100, this represents a very low subjective ambient noise level. However, a significant effect of LBNP on momentary arousal was revealed (F[3,33] = 5.02, p = 0.01, η^2= 0.32), indicating higher subjective arousal with increasing LBNP gradients. Momentary arousal ratings were 38.0 (SEM = 5.2) for 0 mmHg, 34.6 (SEM = 5.7) for -10 mmHg, 41.1 (SEM = 4.8) for -20 mmHg, and 43.0 (SEM = 5.5) for -30 mmHg. Given the scale range of 0 to 100, this represents a low to medium arousal for lower LBNP levels and a medium subjective arousal state for higher LBNP. Mean ratings and standard errors are summarized in Table 2.

Table 2. Ratings of momentary arousal, affective valence, and ambient noise at 0, -10, -20, and -30 mmHg of LBNP.

	LBNP 0 mmHg		LBNP -10 mmHg		LBNP -20 mmHg		LBNP -30 mmHg	
	mean	(SE)	mean	(SE)	mean	(SE)	mean	(SE)
momentary arousal [AU]	38.0	(5.2)	34.6	(5.7)	41.1 *	(4.8)	43.0 *	(5.5)
affective valence [AU]	63.5	(3.7)	62.6	(3.2)	63.0	(3.6)	62.0	(3.2)
ambient noise [AU]	7.6	(2.4)	7.5	(2.0)	7.8	(2.1)	8.1	(2.1)

* Significant differences relative to LBNP of 0 mmHg (based on the within-participant contrasts of the ANOVA with repeated measures design).

5.3.3 Startle Data

Startle magnitude increased with increasing levels of LBNP (F[3,33] = 4.53, p = 0.01, η^2 = 0.29). Subsequent analysis revealed a significant linear trend of pressure gradients on startle magnitude (F[1,11] = 15.4, p = 0.002); quadratic and cubic trends were not significant.Also, a significant main effect of the cardiac cycle phase on startle magnitude was found (F[1,11] = 4.59, p = 0.05, η^2 = 0.29). No significant interaction of LBNP level × cardiac cycle phase could be detected (F[3,33] = 0.05, p = 0.98). A graphical representation of startle amplitude with standard errors for the respective LBNP conditions and cardiac cycle phase is given in Figure 7.

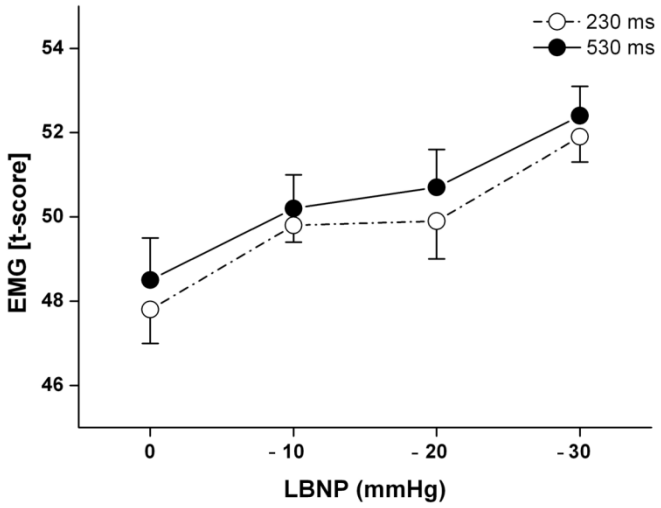

Figure 7. Mean EMG responses to startle stimuli (t-scored) at 0, -10, -20, and -30 mmHg of LBNP. Lines represent early (systolic) cardiac cycle phase (empty circles) and late (diastolic) cardiac cycle phase (filled circles). Error bars represent ±1 standard error of mean.

5.3.4 Reaction Time Data

For the reaction times to startle stimuli, no significant effect of LBNP (F[3,33] = 0.15, p = 0.93) or cardiac cycle phase (F[1,11] = 0.16, p = 0.70) were observed. An interaction of cardiac cycle phase and LBNP on reaction time (F[3,33] = 2.82, p = 0.05, η^2 = 0.20) was present, but disappeared when mean individual reaction time was included as a covariate. A graphical representation of reaction times with standard errors for the respective LBNP conditions and cardiac cycle phase is given in Figure 8.

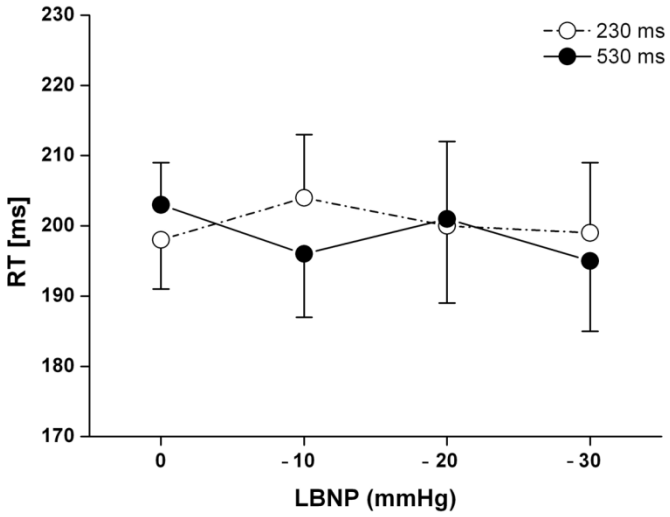

Figure 8. Mean reaction times to startle stimuli at 0, -10, -20, and -30 mmHg of LBNP. Lines represent early (systolic) cardiac cycle phase (empty circles) and late (diastolic) cardiac cycle phase (filled circles). Error bars represent ±1 standard error of mean.

5.4 Discussion

The main aim of the current study was to investigate whether manipulation of cardiopulmonary 'low pressure' baroreceptor activity influences startle responsiveness. The principle new finding of this study is that cardiopulmonary baroreceptor unloading enhances startle eye blink responsiveness in a continuous dose-response fashion (the stronger the unloading, the higher the startle magnitude).

Furthermore, we assessed the effects of relative arterial baroreceptor loading/unloading on startle eye blink responsiveness to indicate feasibility of the research methodology. We could replicate the previously described effect of increased startle eye blink responsiveness by relative arterial baroreceptor unloading. This replication serves as validation of the research approach.

Some previous studies have suggested that loading of arterial baroreceptors can inhibit psychomotor reactions (Edwards et al., 2007; McIntyre et al., 2008b; Stewart et al., 2006; Vaitl et al., 1996), but this study, as well as other studies (Thompson & Botwinick, 1970;

Weisz & Adam, 1996) did not find such an effect. Furthermore, this study did not find an effect of cardiopulmonary baroreceptor unloading on psychomotor reaction time.

Because of the assumed orthogonal variation of cardiopulmonary and arterial baroreceptor activity, we tested whether manipulations of both receptor types would statistically interact with respect to reflexive startle eye blink data, but such effects were not present. No effects of arterial or cardiopulmonary baroreceptor manipulation were found for voluntary psychomotor reaction times in this study, which is well in line with previous research. Three previous studies have addressed potential effects of baroreceptors on higher CNS functions. Two studies (McIntyre, Kavussanu & Ring, 2008a; McIntyre, Ring, Hamer & Carroll, 2007) used fixed coupling of reflex-evoking stimuli to electrocardiogram R-wave occurrence in participants who lay supine with their legs raised (which results in cardiopulmonary baroreceptor loading) or lowered, and showed an inhibitory effect of arterial, but not of cardiopulmonary, baroreceptors on reaction times. However, a further study (Vaitl & Gruppe, 1990) used simulated micro-gravity head-up head-down tilt technology, and found a summative contribution of arterial and cardiopulmonary baroreceptor afferent feedback on EEG theta-band activity, so that the question of baroreceptor influences on higher CNS structures remains unresolved and needs to be investigated by future research, which also may address interactions of arterial and cardiopulmonary baroreceptors. Such interactions have been described in previous research on the level of cardiovascular homeostatic neural processes (Brown et al., 2003; Cooper & Hainsworth, 2001; Victor & Mark, 1985), suggesting that the two baroreceptor systems have the potential to interact in a complex way. This study is not able to address these interactions because of the following limitations: LBNP represents a static unloading of cardiopulmonary baroreceptors, in contrast to a phasic loading and unloading of arterial baroreceptors. Interactions (or absence of interactions) found in this study may, thus, not be generalizable to other static/phasic manipulation patterns. Furthermore, LBNP may affect the temporal dynamics of the cardiac ejection, so that the chosen latencies of stimulus presentation may not be optimal during every LBNP gradient. However, the pre-ejection period and left ventricular ejection time data described in this study argue that the temporal cardiac dynamics of the cardiac ejection are subject to minor changes only. As summarized by Edwards and colleagues (2009), neural baroreceptor activity peaks 100 ms after the pulse pressure wave reaches the sinus carotis, i.e. at R +240ms (Angell James, 1971b; Coleridge et al., 1987). After the pulse pressure wave has passed the

baroreceptors at the sinus carotis, baroreceptor activity quickly decreases (Angell James, 1971a; Coleridge, Coleridge, Poore, Roberts & Schultz, 1984) until baroreceptor afferents are mostly silent after an additional 150 ms (R +390ms) until the onset of the next pulse pressure wave (Chapleau & Abboud, 1987; Koley, Pal & Koley, 1989; Seagard et al., 1990). Accordingly, even at altered interbeat intervals during LBNP, all stimuli should have been appropriately placed.

We observed cardiovascular responses to LBNP which were comparable to previous reports (Cooke, Ryan & Convertino, 2004; Franke et al., 2003; Hinghofer-Szalkay et al., 1996; Kitano et al., 2005; Laszlo et al., 1998; van Hoeyweghen et al., 2001). Thorax impedance increased while stroke volume decreased, reflecting blood pooling in the legs and subsequent reduction of blood volume in the central venous compartment. Systolic blood pressure and interbeat intervals decreased, while diastolic blood pressure increased slightly. These changes reflect the cardiovascular adjustments to LBNP, such as changed cardiac mechanics due to reduced end-diastolic volume and an increased peripheral resistance induced by increasing LBNP. Mean arterial blood pressure remained unaffected by the LBNP intervention, showing that the LBNP procedure can be regarded as a non-hypotensive manipulation of central venous blood volume. The application of LBNP resulted in decreased normalized heart rate variability in the high frequency band, suggesting vagal withdrawal, increased normalized heart rate variability in the low frequency band (Task Force, 1996; Berntson et al., 1997), increased LF/HF ratio (Malliani et al., 1994; Montano et al., 1994; Zaza & Lombardi, 2001), and reduced T-wave amplitude (Contrada et al., 1989; Furedy, Heslegrave & Scher, 1992; Furedy, Szabo & Peronnet, 1996), suggesting increased sympathetic activity. It should be noted that respiratory frequency significantly decreased with increasing LBNP gradients, so that an effect of respiration on heart rate variability cannot be excluded. However, the decrease was rather small (0.03 Hz), and respiratory frequency remained well within the high frequency band. Since a decrease of respiratory frequency is associated with an increase in high frequency heart rate variability (Angelone & Coulter, 1964; Schachinger et al., 1991) (given that the respiratory frequency is greater than 0.1 Hz), an increase in high frequency heart rate variability would be expected due to the respiratory frequency change, but the opposite effect (a decrease) was found. Thus, the induced changes in heart rate variability reflect true parasympathetic withdrawal, serving as a manipulation check in our study, to document the previously described impact of LBNP on autonomic nervous system function

(Franke et al., 2003; Kitano et al., 2005; Laszlo et al., 1998). The cardiovascular and autonomic nervous system changes are well in line with increased arousal ratings observed during increased LBNP levels in this study.

This is the first study to indicate increased startle eye blink responses to acoustic noise stimuli during unloading of cardiopulmonary baroreceptors. Stronger reactions to startle stimuli, either voluntarily, as in the case of psychomotor responses, or automatically, as in the case of reflexive startle eye blink, are protective and potentially adaptive when confronted with hostile environmental demands. LBNP has been introduced as a model for haemorrhagic stress (Convertino et al., 2006; Cooke & Convertino, 2005; Cooke, Rickards, Ryan & Convertino, 2008; Cooke et al., 2004; Rea et al., 1991), and the cardiovascular changes, especially the direction of heart rate variability changes observed in this study, correspond to autonomic function changes seen after moderate blood loss of about 500 ml (Haberthur, Schachinger, Seeberger & Gysi, 2003). Indeed, it is likely that during states of blood loss stress, which may occur as a result of a predator attack, the support of protective mechanisms enhances the organism's chance of survival. Protective startle responses are favoured during negative affect and stress (Grillon, Duncko, Covington, Kopperman & Kling, 2007; Lissek et al., 2007; Mol, Baas, Grillon, van Ooijen & Kenemans, 2007), and were found to correlate positively with cardiovascular stress responses (Gautier & Cook, 1997). On the other hand stressful and attention-demanding tasks may attenuate startle (Neumann, 2002). In the current study, participants did not perceive the application of LBNP as unpleasant. It therefore seems unlikely that the observed increased startle magnitude is mediated by a negative affective state. Rather, our results suggest that baro-afferent processes may play a role in adjusting protective response strategies during stressful circumstances, since unloading of baroreceptors, as would occur during states of blood loss, was associated with an enhancement of protective automatic reactions.

Previous studies have found reaction time, especially the pre-motor 'cognitive' component of psychomotor reactions, to be shorter during unloading of arterial baroreceptors (Edwards et al., 2007; McIntyre et al., 2008b; Stewart et al., 2006), but others have not (Thompson & Botwinick, 1970; Weisz & Adam, 1996). In line with the latter finding, our results did not show an effect of either arterial or cardiopulmonary baroreceptors on reaction times. Hence,

this supports the assumption that automatic protective reactions (such as reflexive eye-blinks) are more likely to be disinhibited during blood loss stress than are voluntary psychomotor reactions, suggesting that 'archaic' automatic brainstem-based protective responses may be favoured during haemorrhagic challenge.

In conclusion, these results demonstrate effects of cardiopulmonary as well as arterial baroreceptors on protective reflexive startle eye blink responses. Our results support the speculation that cardiopulmonary baroreceptors may play a role in adapting response strategies during stressful circumstances, such as favouring automatic brainstem relayed responses, while having no such effect on voluntary psychomotor reactions. Our results, furthermore, suggest that effects on startle need to be taken into consideration in investigations employing procedures which affect cardiopulmonary baroreceptor loading status, i.e. changes in body position or changes in fluid intake or output, or after evoking other forms of central venous blood loss.

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5.ii AUTHOR NOTES

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Erklärung

nach § 9, Abs. 1 der Promotionsordnung des Fachbereichs I der Universität Trier vom 13.11.2008.

Hiermit versichere ich, dass ich die vorliegende Arbeit selber verfasst und keine außer den angegebenen Hilfsmitteln und Referenzen benutzt habe. Die Arbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

André Schulz, Dipl.-Psych. Trier, im Oktober 2009