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The startle response in psychophysiological research:

modulating effects of contextual parameters

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Acknowledgments

General Abstract

Startle reactions are fast, reflexive, and defensive responses which protect the body from injury in the face of imminent danger. The underlying reflex is basic and can be found in many species. Even though it consists of only a few synapses located in the brain stem, the startle reflex offers a valuable research method for human affective, cognitive, and psychological research. This is because of moderating effects of higher mental processes such as attention and emotion on the response magnitude: affective foreground stimulation and directed attention are validated paradigms in startle-related research.

This work presents findings from three independent research studies that deal with (1) the application of the established "affective modulation of startle"-paradigm to the novel setting of attractiveness and human mating preferences, (2) the question of how different components of the startle response are affected by a physiological stressor and (3) how startle stimuli affect visual attention towards emotional stimuli. While the first two studies treat the startle response as a dependent variable by measuring its response magnitude, the third study uses startle stimuli as an experimental manipulation and investigates its potential effects on a behavioural measure.

The first chapter of this thesis describes the basic mechanisms of the startle response as well as the body of research that sets the foundation of startle research in psychophysiology. It provides the rationale for the presented studies, and offers a short summary of the obtained results. Chapter two to four represent primary research articles that are published or in press. At the beginning of each chapter the contribution of all authors is explained. The references for all chapters are listed at the end of this thesis.

The overall scope of this thesis is to show how the human startle response is modulated by a variety of factors, such as the attractiveness of a potential mating partner or the exposure to a stressor. In conclusion, the magnitude of the startle response can serve as a measure for such psychological states and processes. Beyond the involuntary, physiological startle reflex, startle stimuli also affect intentional behavioural responses, which we could demonstrate for eye movements in a visual attention paradigm.

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This doctoral thesis consists of three chapters (and, in addition, one chapters that represent a general introduction and overview), which are published as 'Original Articles' in international peer-reviewed journals. The author of this dissertation is the first author of all three articles. However, other authors also contributed to the work (listed in the table). All articles are presented here in the originally published form, except for changes in formatting (i.e. figure labelling). References for all three articles are provided at the end of this work.

Content   *has been published as:*

Chapter II   Lass-Hennemann, J., **Deuter, C. E**., Kuehl, L. K., Schulz, A., Blumenthal, T. D., & Schachinger, H. (2011):
Effect of facial self-resemblance on the startle response and subjective ratings of erotic stimuli in heterosexual men.
Archives of Sexual Behavior, 40 (5), 1007-1014.
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Chapter III   **Deuter, C. E**., Kuehl, L. K., Blumenthal, T. D., Schulz, A., Oitzl, M. S., & Schachinger, H. (2012):
Effects of Cold Pressor Stress on the Human Startle Response.
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Chapter IV   **Deuter, C. E**., Schilling, T.M., Kuehl, L. K., Blumenthal, & Schachinger, H. (2013):
Startle effects on saccadic responses to emotional target stimuli.
Psychophysiology, 50 (10), 1056–1063
DOI: 10.1111/psyp.12083

Index of Abbreviations

Ag/AgCl	Silver/Silver chloride
ANOVA	Analysis of variance
ANS	Autonomic nervous system
bpm	Beats per minute
BP	Blood pressure
C	Celsius
cm	Centimeter
dB	Decibels
DBP	Dystolic blood pressure
e.g.	for example
ECG	Electrocardiogram
EMG	Electromyography
ER	Error rate
HR	Heart rate
Hz	Hertz
IAPS	International affective picture system
i.e.	that is
M	Mean
min	Minutes
mm	Millimeter
mmHg	Millimeters mercury
ms	Milliseconds
neg	negative
ntr	neutral
OOM	Orbicularis oculi muscle
pos	positive
px	Pixel
s	Seconds
SCR	Skin conductance response
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
SEM	Standard error of mean

Chapter I − General Rationale

What can a simple physiological reflex contribute to psychological research? Ever since Eric Kandel fundamentally revolutionized our understanding for learning and memory by studying the gill-withdrawal reflex in Aplysia (Kandel & Squire, 2000), the answer must be: quite a lot! In a similar vein, the basic startle reflex provides psychologists with a simple systems model for the research on attention and emotion. Over recent decades, the measurement of the human startle response has proven to be a useful and viable method for psychophysiological research (Blumenthal et al., 2005). Even though the startle response consists of a simple reflex circuit with a small number of connections, it is highly susceptible to contextual influences. Physiological, as well as psychological, states of the organism modulate the response. The response modulation due to attentional and affective processes has spawned a vast amount of research work. Both influences have initially been assessed as separate aspects; however, it soon became obvious that attention and affect can interact in intricate ways.

Whether the individual components of the startle response form a homogeneous system is not fully elucidated. The contraction of several skeletal and facial muscle systems coincides with a brief surge in autonomic sympathetic activation. Because of its slow habituation rate and ease of measurement, psychophysiological research usually measures the orbicularis oculi muscle (OOM), which directly contributes to the startle related eye blink (Blumenthal, et al., 2005). However, the relation between OOM activation and blink kinematics on the one hand, and autonomic parameters of the response on the other, could be susceptible to state variables such as stress.

Another line of research that addresses startle is focussing on voluntary movements, instead of involuntary reflexes, as a dependent variable. In reaction time tasks, responses are accelerated when they coincide with or are shortly preceded by a startle stimulus (Valls-Sole, Kumru, & Kofler, 2008). How the so-called 'StartReact' effect relates to the reflexive startle response and whether affective states have a modulating effect is a matter of ongoing debate.

The aim of this thesis is to highlight these modifying processes even further. With affective modulation of the startle response as an underlying theme, these empirical studies bring together different physiological and psychological factors and their influence on various response parameters. The presented body of research investigates the influence of physiological stress on the startle response, whether acoustic startle probes can influence visuospatial attention to emotional target stimuli and whether the established paradigm of affective startle modulation can be applied to the context of mate preferences.

1.1 The startle response

The startle response is a fast, defensive reaction to a sudden and intense stimulus. The response is highly functional adaptive and its components are manifold. Still, they all converge on one purpose: to cope with an imminent threat. Three different aspects of the response can be distinguished. Fast, generalized muscle contractions induce a secure body posture and protect vulnerable structures, such as the neck or the eye ball (Koch, 1999). Beyond this immediate physical protection, a brief surge in autonomic activation prepares for a flight-or-fight response. This vegetative component of the startle response can be measured in different parameters, such as heart rate or skin conductance (Holand, Girard, Laude, Meyer-Bisch, & Elghozi, 1999; Lipp, Siddle, & Dall, 1998). Finally, ongoing cognitive processes are disrupted and attention is rapidly reoriented (Herbert & Kissler, 2010; Miller, Patrick, & Levenston, 2002).

The discovery of the startle response dates back to the 19th century, when the Russian physiologist Ivan Sechenov first described it in his book "Reflexes of the brain" (Sechenov, 1863). However, this discovery did not generate any further interest until 1939, when the response pattern of animals and humans was cinematically recorded (Landis & Hunt, 1939). Again, the research on startle fell into oblivion until the incidental discovery of fear-conditioned startle (Brown, Kalish, & Farber, 1951), an early manifestation of affective modulation, in which startle is elicited in the presence of a stimulus that has previously been paired with fear evoking stimuli. Realizing that the startle response is susceptible to contextual and organism variables, this study of Brown et al. unleashed a vast amount of research, mainly by Hoffman and colleagues (Hoffman & Fleshler, 1963; Hoffman & Searle, 1965; Hoffman & Wible, 1969).

1.2 Affective startle modulation

When Brown et al. (Brown, et al., 1951) investigated classical conditioning of fear in rats, one experimental design paired a neutral stimulus with electric shocks. In some trials, this neutral/conditioned stimulus was followed by an acoustic startle stimulus. This led to higher startle magnitudes in the experimental group, when compared to the unconditioned control group. Then unaware of the potential implications, Brown wrongfully interpreted increased startle as an energizing function of conditioned fear. In the following decades, fear-potentiated startle became a topic of growing research interest, with studies investigating modes of timing (Davis, Schlesinger, & Sorenson, 1989; Grillon, Ameli, Merikangas, Woods,

& Davis, 1993), or the underlying neural circuitry (Davis, 1986; Davis, Falls, Campeau, & Kim, 1993). The coupling of fear to a previously neutral stimulus was the first implementation of startle modification by affect.

This restriction to fear-conditioning was overcome with the discovery of the reverse effect: startle inhibition due to positive affect. The first study to demonstrate this effect exposed participants to acoustic startle probes during the presentation of pleasant images (Simons & Zelson, 1985). Neutral slides of household objects and erotic images taken from men's magazines were used as visual lead stimuli. When acoustic startle probes were delivered with stimulus onset asynchrony (SOA) of 3.5-6s, startle magnitude was on average lower for the erotic slides. The authors interpreted their finding in terms of attentional allocation: with the erotic slides being of higher interest to the participant, the locus of attention was increasingly shifted to the visual domain.

Furthermore, an interpretation in terms of arousal was rejected: based on previous research (Graham, 1979), they argued that increased arousal would facilitate startle, instead of inhibiting it. This position was substantiated by another study in the same year, which could demonstrate that startle magnitude was primarily influenced by allocation of attentional resources (Anthony & Graham, 1985). However, if an aversive, threatening stimulus is just as significant as a pleasant stimulus in terms of attentional allocation, this should result in decreased startle for both - assuming that they are presented in a modality other than that of the startle stimulus. Previous studies in animals, that made use of the fear-potentiation paradigm, reported increased startle to aversive visual foreground stimuli (Berg & Davis, 1984, 1985). In an attempt to resolve this inconsistency, emotional picture sets of positive, neutral and negative were presented to human participants (Vrana, Spence, & Lang, 1988). This study was the first to show that startle magnitude was clearly modulated by the valence dimension of emotions, independent of any attentional manipulations. Positive pictures generated the lowest, negative pictures the highest startle magnitudes, with neutral pictures ranging in the middle. In addition, this was also the first study that applied the recently developed 'International Affective Picture System' (IAPS) to startle research (Lang, Ohman, & Vaitl, 1988), a catalogue of images that should soon become a standard for affective startle modulation.

With the concept of 'motivational priming', the theoretical framework for affective startle modulation was developed (Bradley, Cuthbert, & Lang, 1990; Lang, Bradley, & Cuthbert, 1990). Assuming that emotion reflects a "readiness for action" (Frijda, 1987), negative emotions will activate defensive, avoidance-related response systems while positive

emotions activate appetitive, approach-related responses. The startle probe was conceptualized as a defensive, slightly negative stimulus. When this startle probe is preceded by foreground stimulus, either an approach- or avoidance response will be primed, depending on the valence of the stimulus. In case of a congruency in response motivation, i.e. when the foreground is negative, the resulting response is facilitated; a mismatch following positive stimuli will result in an attenuation of the response.

1.3 Study I: Startle Modulation as a measure of mating preferences in humans

Considering that startle response magnitude in modulated by the valence of foreground stimuli, startle magnitude can be used as a measure of emotional assessment (Mauss & Robinson, 2009). When studying human mating behaviour, the question arises of what contributes to the attraction of a potential mate. One such factor that was found to have an influence is facial similarity. Cues of kinship are predicted to increase pro-social behaviour due to the benefits of inclusive fitness, but to decrease sexual approach motivation due to the potential costs of inbreeding. Previous studies have shown that facial resemblance, a putative cue of kinship, increases pro-social behaviour (DeBruine, 2002). However, the effects of facial resemblance on mating preferences are equivocal, with some studies finding that facial resemblance decreases sexual attractiveness ratings, while other studies show that individuals choose mates partly on the basis of similarity.

Most studies that assessed attractiveness of potential mates relied on subjective ratings, a measure that might be susceptible to demand characteristics and does not capture preferences of which the subject is not aware. Assuming that differences in sexual approach motivation to erotic pictures will modulate startle, we used the paradigm of affective startle modulation. Male participants were confronted with images of erotic female nudes and neutral pictures. To assess facial similarity, the erotic images were digitally altered via a morphing procedure to increase similarity to the participant (who was unaware of this manipulation), or to another random male face respectively. Participants showed greater startle inhibition to self-resembling than to other-resembling or non-manipulated female nude pictures, but subjective pleasure and arousal ratings did not differ between the three erotic picture categories. Our data suggest that visual facial resemblance of opposite-sex nudes increases sexual approach motivation in men, and that this effect is not due to their conscious evaluation of the erotic stimuli.

With this study we could demonstrate a potential application of affective startle modulation. Because conscious evaluation of attitudes and evaluations is limited and self report might be biased, implicit measures of emotions complement the psychologist's toolbox (De Houwer, Teige-Mocigemba, Spruyt, & Moors, 2009). Affective startle modulation can be used to assess such attitudinal processes, even when the participant in unaware of them. This could be successfully demonstrated in this study.

1.4 Generalization across stimuli and modalities

This basic pattern, the linear association of the startle reflex with valence, could be replicated in many studies, using diverse stimuli of different modalities, such as emotional images (Yartz & Hawk, 1999), words (Herbert, Deutsch, Sütterlin, Kübler, & Pauli, 2011), brand logos (Walla, Brenner, & Koller, 2011), music (Roy, Mailhot, Gosselin, Paquette, & Peretz, 2009), films (Kaviani, Gray, Checkley, Kumari, & Wilson, 1999) or odours (Miltner, Matjak, Braun, Diekmann, & Brody, 1994; Pause, Adolph, Prehn-Kristensen, & Ferstl, 2009). Nevertheless, some caveats to the affective modulation of startle were discovered in the years following. The degree of affective modulation is clearly a function of arousal, it works best with foreground stimuli that are high in arousal (Cuthbert, Bradley, & Lang, 1996). This is important, considering the unequal distribution of stimuli in the 'affective space' (Lang, Bradley, & Cuthbert, 1999).

Furthermore, the linear function of modulation by valence might not be unequivocal for all contexts and settings. For instance, while studies that used imagery of emotional scenes reported startle facilitation with negative imagery, the attenuation effect for positive scenes was absent (Robinson & Vrana, 2000) or even reversed: positive scenes facilitated startle (Miller, et al., 2002; vanOyen Witvliet & Vrana, 2000). Similar results were reported for the processing of emotional words: positive words increased startle responses (Herbert, Kissler, Junghöfer, Peyk, & Rockstroh, 2006), an effect that might be moderated by the level of processing (Herbert & Kissler, 2010). These results raise doubt about the generalizability of the 'motivational priming' hypothesis. With "processing interrupt", another mechanism has been suggested as an explanation for these findings: during modes of internally directed processing, startle stimuli disrupt these processes and refocus attention to the external environment. For internal processing, this mechanism could override "motivational priming" and reverse the effect which would be predicted for valence matching (Herbert & Kissler, 2010; Miller, et al., 2002).

1.5 Measurement and physiological foundations of the startle response

The startle response cascade follows a unique pattern of facial and skeletal muscle activation (Landis & Hunt, 1939). The muscle with the shortest reaction latencies, and therefore the first in the cascade, is the orbicularis oculi muscle (OOM), with median response latencies of only 36.7ms (Brown et al., 1991). Contraction of the OOM results in an eye blink, thereby covering the sensitive eyeball and protecting it from injury. Because of its short latency, but also because it is the most reliable response and most resistant to habituation, the startle eye blink response is the most common response measure (Blumenthal, et al., 2005). Electromyographical recording (EMG) was early described to be a practical and economical assessment tool of the response (Jones & Kennedy, 1951). Since then it has gained widespread acceptance and is a research standard by now. Because of reliable elicitation, the most commonly used stimulus type to elicit startle responses in humans is an acoustic, white-noise probe of high intensities and short duration (Blumenthal, et al., 2005).

The primary acoustic startle pathway is restricted to only three synapses, with the pontine reticular formation as a central integrating structure. In particular, the nucleus reticularis pontis caudalis (nRPC) receives auditory input from cochlear root neurons. From there on, activation is conducted to the facial motor nucleus (for facial muscles) and via the spinal cord to the motor neurons of the relevant muscles (Davis, 2006; Yeomans & Frankland, 1995). The nRPC receives input from various other brain structures that can modulate the reflexive response at this stage. Relevant structures for affective startle modulation are the amygdala and, for inhibition due to pleasant stimuli, the nucleus accumbens (Grillon & Baas, 2003).

1.6 Study II: Effects of stress on the startle response

Stress is regarded to be an adaptive reaction to an adverse stimulus or situation. The stress response is a multi-level, complex shift in the organism's physiological and psychological functioning (Del Giudice, Ellis, & Shirtcliff, 2010). Physiological stress allocates bodily resources to facilitate quick, evasive actions at the expense of more long term, regenerative functions. Acute stress involves an endocrinal response (de Kloet, 2003) , activation of the sympathetic nervous system (Ulrich-Lai & Herman, 2009), and influences somatic motor behaviour and psychological adjustments. In contrast to the low-level, biological adaptations, that meet the change in energy demands, stress effects on basic psychological processes, and interaction with attention and emotion, are less well understood.

Previous studies found an attentional bias for aversive, threatening stimuli under stress exposure (Chen, Lewin, & Craske, 1996; Mogg, Mathews, Bird, & Macgregor-Morris, 1990), while others found no (McNally, Riemann, Louro, Lukach, & Kim, 1992; Mogg, Kentish, & Bradley, 1993) or even opposing effects, with less attention for negative stimuli after stress manipulation (Ellenbogen, Schwartzman, Stewart, & Walker, 2002; Mathews & Sebastian, 1993).

Several studies assessed the effect of cortisol, an endocrinological measure related to stress coping, on the human startle response. The results are somewhat contradictory. Making use of the diurnal variation of cortisol excretion, one study assessed startle responses during different daytimes: responses were increased with low cortisol levels and decreased when levels were high (Miller & Gronfier, 2006). Corresponding to this finding, a pharmacological suppression of cortisol levels enhanced startle responses (Roemer, Nees, Richter, Blumenthal, & Schächinger, 2009). However, intravenous applications of cortisol revealed either no effect on startle responses (Richter, Schulz, et al., 2011), or reported an inverse U-shaped relation: small doses (5mg) increased and higher doses (20mg) decreased startle responses (Buchanan, Brechtel, Sollers, & Lovallo, 2001).

In study II, we assessed the influence of an acute physiological stressor on the startle response. From the fact that stress activates the defensive, avoidance-related system similar to unpleasant stimuli, we hypothesized that acute stress facilitates the startle response. While studies that investigate the effects of affective startle modulation commonly use brief presentation of stimuli, subjects were exposed to the unpleasant stressor for three minutes. In addition to the well-established eye blink response we also assessed various autonomic startle measures. Assuming that autonomic components of the response are more related to activation instead of protection, these components were expected to show even higher susceptibility to the stress intervention.

We used an established physiological stressor, the Cold Pressor Test (CPT), to induce stress in a non-clinical human sample (24 student participants) in a within-subjects design. Autonomic (heart rate and skin conductance) and somatic (eye blink) responses to acoustic startle probes were measured during a pre-stress baseline, during a three minutes stress intervention, and during the subsequent recovery period. Startle induced skin conductance responses and heart rate responses were facilitated during stress. Compared to baseline, startle eye blink responses were not affected during the intervention but were diminished afterwards. These data describe a new and unique startle response pattern during stress: facilitation of autonomic stress responses but no such facilitation of somatic startle eye blink responses.

1.7 Influences of attention and lead stimulus interval

Another aspect by which startle is modulated is attention. When the affective modulation of startle was described first, it was soon suggested that emotion is only superficially responsible for this effect. The driving force would be attentive processes: emotional pictures plainly capture more attention (Simons & Zelson, 1985). Even though it was soon demonstrated that affective modulation was a phenomenon in itself (Bradley, et al., 1990; Lang, 1995), subsequent research could also show that emotion and attention interact in startle paradigms. Several studies could demonstrate that startle responses were enhanced when attention is directed to the foreground stimulus, independent of its valence (Lipp, Neumann, Pretorius, & McHugh, 2003; Lipp, Siddle, & Dall, 1997; Mallan, Lipp, & Libera, 2008; Neumann & Lipp, 2003). Again, this effect was modulated by attentional (Adam, Mallan, & Lipp, 2009) and perceptual load (Thorne, Dawson, & Schell, 2006). However, actively attending the startle probe diminished the effect of affective modulation (Panayiotou, Witvliet, Robinson, & Vrana, 2011). Startle is facilitated when stimuli are attended that match the modality of the startle eliciting stimulus, compared to a mismatch (Anthony & Graham, 1985). It is assumed that selectively focussing attention to a certain modality primes this sensory pathway and facilitates further input in this modality (Filion, Dawson, & Schell, 1998). Still, when attentional focus and affective valence are systematically varied, attentional modulation was overridden by affective modulation (Haerich, 1994). Summarizing these studies, emotion and attention exert a combined influence on the startle response. While affective and attentional startle modulations are independent phenomena, both factors interact under certain conditions.

Affective modulation due to motivational priming was originally obtained for comparatively long lead intervals, e.g. the foreground stimulus was presented for 3-6 s before startle onset (Bradley, Cuthbert, & Lang, 1999). Startle responses are diminished when the probe is preceded by a non-startling stimulus at short lead intervals, e.g. below 300ms. This phenomenon was termed "prepulse inhibition" and has its maximum at a stimulus onset asynchrony (SOA) of 120ms (Norris & Blumenthal, 1996). This phenomenon however is not independent of affective content. An early study examined the influence of affective pictures at short lead intervals (Bradley, Cuthbert, & Lang, 1993). The interval for maximum inhibition was reached at 300ms, somewhat later compared to simple auditory prepulses. This inhibition was stronger for emotional pictures. Valence had no influence: inhibition was equally strong for both pleasant and unpleasant pictures. With higher arousal evoked by the emotional pictures, this effect was seemingly driven by attentional capture.

However, later studies could demonstrate effects of valence at short intervals (Gard, Gard, Mehta, Kring, & Patrick, 2007; Stanley & Knight, 2004). These findings indicate that approach-avoidance related, motivational systems might be active at comparatively short intervals.

1.8 Effects of startle stimuli on voluntary motor reactions

Beyond the reflexive muscle contractions that constitute the startle response, startle stimuli can also affect voluntary reactions. In reaction tasks that require motor responses to an imperative stimulus, responses are accelerated when the imperative stimulus is accompanied or shortly preceded by a simultaneous startle stimulus, a phenomenon that is termed "StartReact" (Valls-Sole, et al., 2008). This acceleration is most pronounced in simple reaction time task, i.e. those where the response that is to be executed is known beforehand and can be "pre-planned" (Carlsen, Maslovat, Lam, Chua, & Franks, 2011). However, StartReact has also been demonstrated in choice-reaction time tasks, those in which the correct response is indicated by the imperative stimulus (Oude Nijhuis et al., 2007; Reynolds & Day, 2007). While originally demonstrated with manual response tasks, the StartReact phenomenon could also be shown for saccadic reactions (Castellote, Kumru, Queralt, & Valls-Sole, 2007). How the StartReact-effect relates to the involuntary startle response and if the startle response magnitude is indicative for the accelerating effect remains controversial (Carlsen, Dakin, Chua, & Franks, 2007; Maslovat, Kennedy, Forgaard, Chua, & Franks, 2012; Reynolds & Day, 2007).

1.9 Study III: Startle effects on saccadic responses to emotional target stimuli

Even though a growing body of research is dedicated to startle influences on voluntary reactions, it remains unclear how this is modulated by the affective valence of target stimuli. Furthermore, affective startle modification with visual stimuli has primarily been demonstrated with directly attended images. This study investigated the effect of acoustic startle stimuli and target valence on saccade reaction times and error rates. Saccadic eye movement to specified target locations are influenced by the affective valence of these visual targets (Calvo & Nummenmaa, 2007; Calvo, Nummenmaa, & Hyona, 2008; Gutierrez & Calvo, 2010; Kissler & Keil, 2008; Nummenmaa, Hyona, & Calvo, 2006, 2009). In a reaction task that directly compared saccadic and manual responses to emotional faces, both

measures were affected by target emotionality. However, saccades showed an emotional bias at substantially shorter latencies (Bannerman, Milders, & Sahraie, 2009).

In this study, participants had to perform saccades towards (pro-saccade) or away (anti-saccade) from target pictures that appeared parafoveally, i.e. outside of the focus of visuo-spatial attention. The task in this study was a choice-reaction task: whether a pro- or anti-saccade had to be performed was indicated by an imperative stimulus at central location. Startle probes were presented in two time interval conditions: 500ms prior to and simultaneous with an imperative stimulus, after which saccades to positive, neutral, or negative peripheral target pictures had to be performed. We found that startle stimuli at 500ms SOA reduced reaction times and error rates. However, the effect was independent of target valence or saccade direction. Valence interacted with saccade direction: pro-saccades to negative and anti-saccades to positive targets were delayed.

Startle was shown to speed saccadic reactions and improve accuracy when presented with sufficient lead time before an imperative stimulus. Furthermore, we could demonstrate that emotional information of parafoveally presented images has a moderating influence on prepulse inhibition, perhaps due to increased arousal of those images. The results do not provide support for a valence dependent effect of startle on oculomotor reactions. While startle responses are affected by emotional foreground stimuli, responses to emotional stimuli seem to be unaffected by previous startle stimuli. Independent of the startle related effects, we found a remarkable interaction pattern between saccade direction and target valence which suggests that approach-avoidance motivation can be extended to gaze behaviour.

Chapter II: Effect of facial self-resemblance on the startle response and subjective ratings of erotic stimuli in heterosexual men.

Authors: Johanna Lass-Hennemann, Christian E. Deuter, Linn K. Kuehl, André Schulz, Terry D. Blumenthal & Hartmut Schächinger.

2.0 Abstract

Cues of kinship are predicted to increase prosocial behavior due to the benefits of inclusive fitness, but to decrease sexual approach motivation due to the potential costs of inbreeding. Previous studies have shown that facial resemblance, a putative cue of kinship, increases prosocial behavior. However, the effects of facial resemblance on mating preferences are equivocal, with some studies finding that facial resemblance decreases sexual attractiveness ratings, while other studies show that individuals choose mates partly on the basis of similarity. To further investigate this question, a psychophysiological measure of affective processing, the startle response, was used in this study, assuming that differences in sexual approach motivation to erotic pictures will modulate startle. Male volunteers (n = 30) viewed 30 pictures of erotic female nudes while startle eyeblink responses were elicited by acoustic noise probes. The female nude pictures were digitally altered so that the face either resembled the male participant, another participant, or were not altered. Non-nude neutral pictures were also included. Importantly, the digital alteration was undetected by the participants. Erotic pictures were rated as being pleasant, and clearly reduced startle eyeblink magnitude as compared to neutral pictures. Participants showed greater startle inhibition to self-resembling than to other-resembling or non-manipulated female nude pictures, but subjective pleasure and arousal ratings did not differ between the three erotic picture categories. Our data suggest that visual facial resemblance of opposite-sex nudes increases sexual approach motivation in men, and that this effect is not due to their conscious evaluation of the erotic stimuli.

Keywords: facial self-resemblance, kin recognition, inbreeding avoidance, erotic stimuli, affective startle modulation

2.1 Introduction

Humans and many other vertebrates are able to recognize their kin. Experimental evidence shows that humans are able to recognize genetic similarity based on shared olfactory and visual cues (Oda, Matsumoto-Oda, & Kurashima, 2006; Wedekind, 2007; Weisfeld, Czilli, Phillips, Gall, & Lichtman, 2003). Kin recognition is important, because organisms can increase their fitness by recognizing and responding appropriately to kin. Such responses are said to be context dependent, because the fitness-enhancing response to kin is different in the context of nepotism and mate choice: Cues of kinship are predicted to increase non-sexual prosocial regard owing to the benefits of inclusive fitness (Hamilton, 1964) while decreasing sexual approach motivation owing to the costs of inbreeding (Bateson, 1983; Bittles & Neel, 1994). Indeed, several studies have shown that experimentally induced facial resemblance, a putative cue of kinship, increases prosocial attributions like trusting behavior (DeBruine, 2002), self-reported preference for children (Debruine, 2004b; Platek, Burch, Panyavin, Wasserman, & Gallup, 2002; Platek et al., 2003), and the attractiveness of same-sex faces (DeBruine, 2004).

While there is considerable evidence for increased prosocial attributions for self-resembling faces, the evidence for the effects of kinship cues on mating preferences is less clear. Experimental manipulations of facial resemblance have shown that self resembling opposite-sex faces (putative mating partners) are not rated to be more attractive than control faces (DeBruine, 2004). Extending these findings, DeBruine (2005) showed that self-resemblance increased attributions of trustworthiness to opposite-sex face images, but had no effect on attractiveness in the context of a long-term relationship, and decreased attractiveness in the context of a short-term (sexual) relationship. These findings support the notion that cues of kinship (facial similarity) decrease sexual approach motivation.

On the other hand, observational studies have shown that human romantic partners tend to resemble each other in many traits, including facial characteristics (Bereczkei, Gyuris, Koves, & Bernath, 2002; Bereczkei, Gyuris, & Weisfeld, 2004). Imprinting-like mechanisms have been suggested to account for this effect (Bereczkei, et al., 2002; Daly, 1989): Cross-fostering studies with animals and adoption studies with humans have revealed that animals and humans prefer sexual partners that are similar to the opposite sex-parent that reared them (Immelmann, Pröve, Lassek, & Bischof, 1991; Oetting, Pröve, & Bischof, 1995). This is believed to be due to a fixation to a set of family characteristics that later shape mate preferences during adulthood.

Thus, there is a lack of consensus as to whether facial similarity decreases the sexual approach motivation to potential mating partners or increases it. One problem inherent in the previously described studies is that their designs are solely based on subjective ratings of faces, which might not be the optimal method for analyzing mating preferences, e.g. sexual approach motivation to potential mates. The validity of subjective ratings as a measure of emotional and motivational status is problematic for several reasons. First, subjective ratings require introspection, which is susceptible to error, reconstruction, and inaccuracy (Nisbett & Wilson, 1977). Second, factors such as demand characteristics and social desirability may play a role in the answer tendencies. Third, there is often a considerable time gap between the affective process itself (for example, during viewing of the picture) and the retrieving of the affective process (since the subjective ratings are usually given after picture viewing) during which the impression of the target can decay or be influenced by new input. Furthermore, the use of face stimuli alone is not optimal for analyzing mating preferences. Studies that focus on aspects of sexual approach motivation and states of sexual arousal typically use erotic pictures or film segments as stimulus material and, in addition to subjective ratings, use physiological measures as dependent variables.

One well validated and widely used physiological method for assessing affective valence in the laboratory is the affective startle modulation paradigm. Numerous studies have shown that the startle reflex (elicited by a brief burst of noise) is facilitated when people view aversive pictures and inhibited when people view pleasant pictures (for a review see Bradley, Cuthbert, & Lang, 1999). Viewing of aversive pictures leads to an activation of the defensive system and, therefore, leads to an augmentation of the congruent defensive startle reflex. Viewing of pleasant pictures, on the other hand, engages the appetitive/approach system and leads to an inhibition of the non-congruent defensive startle reflex. Thus, affective startle modulation is not a direct measure of the physiological mechanisms underlying emotion, but rather an indirect measure of the activity of neurobiological structures involved in the processing of approach and withdrawal motivation. The inhibition of the startle reflex while viewing pleasant pictures reflects the activity of neurobiological circuits involved in approach motivation.

Affective startle modulation has been shown to generalize to a variety of foreground stimuli other than pictures that modify the emotional state of the participants (Bradley, et al., 1999), and eliciting the startle response during foreground presentation of different stimuli serves as a validated measure of the affective valence of the presented stimuli. In particular, the startle modulation paradigm has been frequently used to analyze sexual approach

motivation to erotic pictures and film segments (Koukounas & McCabe, 2001; Koukounas & Over, 2000; Lass-Hennemann, Schulz, Nees, Blumenthal, & Schachinger, 2009; Prause, Janssen, & Hetrick, 2008).

The utility of startle over self-report in studying sexual approach motivation is based on the fact that the affective startle modulation paradigm is not hampered by the same problems as subjective ratings are. Affective startle modulation is largely independent of instructions and does not require an introspective focus. It is not a conscious or controlled process and there is a very short time gap between the affective process itself and the measurement of the affective process. Therefore, affective startle modulation appears to be an appropriate measure to evaluate differences in the sexual attractiveness of, and sexual approach motivation to, photographs of erotic female nudes who resemble the participant compared to photographs of erotic female nudes who do not resemble the participant.

In the present study, we investigated whether the extent to which computer manipulated pictures of erotic female nudes that resembled the participant influenced the perceived attractiveness (via subjective ratings) and the sexual approach motivation (via affective startle modulation) of the participant. Participants viewed pictures of erotic female nudes whose facial characteristics were either computer-modified to resemble themselves, or made to resemble another person, or were not manipulated, as well as viewing neutral pictures. We predicted that the startle response in the presence of erotic pictures would be influenced by facial self-resemblance, indicating differences in the activity of neurobiological structures involved in approach motivation.

2.2 Methods

2.2.1 Participants

Participants were 40 male heterosexual students at the University of Trier, Germany, who responded to notices offering 20 € for taking part in two different experiments. Participation was limited to heterosexual Caucasian students without beards, piercings, or tattoos in the facial region. Furthermore, only participants with normal or corrected to normal vision and no history of hearing problems were included in the study. Exclusion criteria were determined by a telephone screening interview. All participants signed a written informed consent and were given a small financial compensation (5 € for the photograph acquisition, 15 € for the actual experiment) for taking part in the experiment. Study procedures were approved by the local ethics committee.

2.2.2 Materials and Design

Experimental material consisted of 40 pictures, 30 of which showed erotic female nudes with a completely visible face (i.e. no hair covering parts of the face), a direct gaze at the observer, and a neutral facial expression. The other 10 pictures were neutral pictures selected from the International Affective Picture System[1] (household objects) (Lang, Bradley, & Cuthbert, 1999).

We used computer imaging techniques to manipulate facial resemblance between pictures of the face of each participant and the faces of the pictures of the erotic female nudes. The pictures of the erotic female nudes formed the basis for the morphing procedure, in which the face of the participant was morphed into the face of the erotic female nude (see Figure 1). Templates were created that specified the contours and certain landmarks on each face. The morphing routine itself comprises two processes: a shape-morph averages the distance between the features of both faces that were specified in the template and a color morph averages the colors of each pixel. These processes result in two different output images, and the color morph was used as a layer on top of the shape morph. To ensure that the resulting female nude pictures still looked attractive and feminine, transparency was added to the color morph layer to a 30% degree. That means that the morphed picture shared 50% of the shape of the participants' face, but only 30% of the color information. By doing this, a composite face was created which was in favour of the women's features, i.e. the face was still primarily female and fitted to the body of the erotic female nude, even though it had a subtle resemblance to the participant. For further details on the underlying technology see Tiddeman, Perrett and Burt (2001). No participants reported detecting the nature of the experiment, suggesting that the morphing did not result in conscious recognition of their own face by any participant.

The 30 pictures of the erotic female nudes were randomly assigned to three subsets that consisted of 10 erotic female nudes each. For each participant, one of the three subsets was morphed with his own face and formed the "self-resembling" erotic female nude picture set, so that ten pictures had a subtle resemblance to the participant. Ten other photos of erotic female nudes were morphed with the face of a different participant and formed the "other-resembling" erotic female nude picture set. The remaining ten female nude pictures remained in their original state and were used as the "not manipulated" erotic female nude picture set. For the assignment of the picture sets to the participants we employed a cross-over design:

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[1] IAPS picture numbers used in this study were 7000, 7002, 7004, 7006, 7009, 7010, 7020, 7025, 7035, 7040

Pairs of two participants were presented with the same version of the picture sets, so that the picture set that formed the self-resembling erotic female nude picture set for participant A formed the other-resembling erotic female nude picture set for participant B and vice versa. That is, every morphed picture set functioned once as the self-resembling erotic female nude picture set and once as the other-resembling erotic female nude picture set.

We included the not manipulated erotic female nude pictures as a control condition to test whether morphed erotic female nude pictures led to a comparable startle inhibition as not manipulated erotic female nude pictures. Furthermore, we included neutral pictures as a control to illustrate the effect of affective startle modulation. Pictures were displayed on an LCD computer and picture onset was virtually instantaneous.
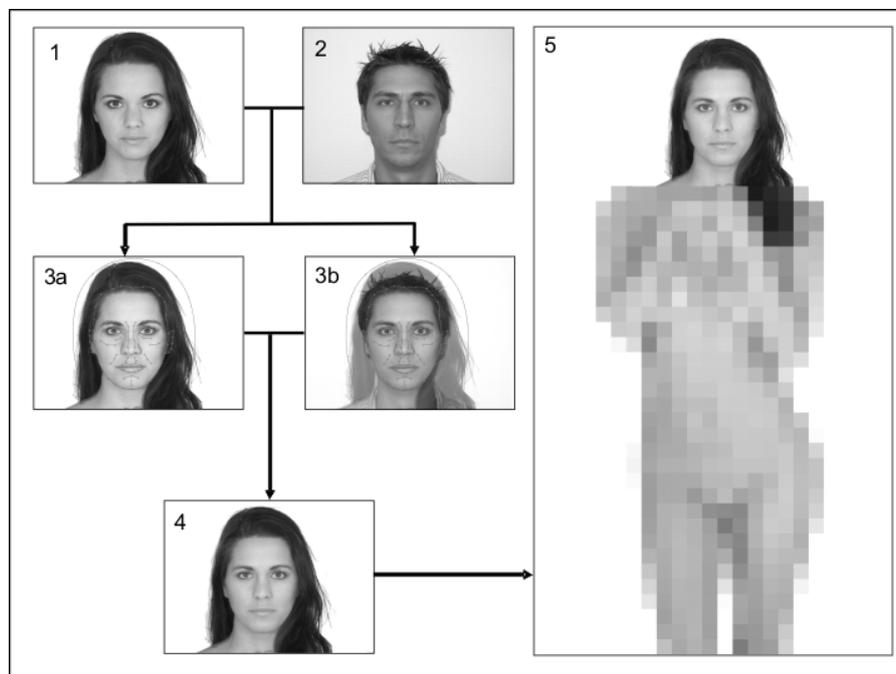
Figure 1: Image editing procedure: The nude woman's detailed face (1) was morphed with the portrait picture of the participant (2). The morphing software produces two output images, a shape-only morph (3a) and a combined shape-color morph (3b). In a second step the shape-color morph is used as a semi-transparent layer on top of the shape-only morph. All artefacts of the morphing procedure are eliminated. The resulting image (4) was photo-mounted on the woman's body in a last step (5). The resulting image was used as a stimulus (the image was not masked in the experiment).

2.2.3 Cover Story

To be able to morph the participants faces into the faces of the erotic female nudes, we had to gather a standardized photograph of each participant's face. For the nature of the experiment it was necessary that participants did not know about the true purpose of the experiment. To assure this, we used the following cover story: Participants were told that for a payment of 20 € they could participate in two unrelated studies in our lab: The experimenter

told participants that the first study was to establish a new emotional face data base that would be used for psychophysiological research in our lab. The second study would analyze emotional and physiological reactions to erotic pictures. Furthermore, the experimenter explained that both studies were run together for economic reasons.

2.2.4 Procedure

Photograph Acquisition. If the participant met the criteria in the telephone interview he was invited to our laboratory to take the photographs of his face. After arriving at the laboratory the experimenter asked the participant to sign an informed consent in which the participant approved the scientific usage of the photographs of his face.

The experimenter instructed the participant to look into the camera with a neutral facial expression. Then the experimenter took the portrait photographs with a digital camera from a distance of 80 cm in a fully lit room.

Startle Pretest. Directly after the photographs were taken, participants underwent a brief startle paradigm. The experimenter explained to the participants that the reaction to startle probes was the main dependent measure in the second study and that some people do not respond to these startle probes. Furthermore, he explained that only those people who showed a reliable startle reaction to the stimuli would be able to participate in the second study. The experimenter attached electrodes for electromyographic (EMG) measurement of the left musculus orbicularis oculi below the left eye of the participant with an inter-electrode distance of 1.5 cm (center to center). A third electrode taped on the forehead served as a reference. Electrode placement and skin preparation followed published guidelines (Blumenthal, et al., 2005). Ten startle probes (105 dB, 50 ms, instantaneous rise time) were presented to each participant and the EMG response to the startle probes were recorded.

After finishing the startle pretest, an appointment for the second part of the study was made with the participants. 10 participants showed less than 70% measurable startle responses and were therefore not invited to the second part of the study. Every participant received 5 Euros for participation and left the laboratory.

Affective Startle Modulation Paradigm. Participants arrived for the second appointment about one week after the pictures were taken. During this week participants' pictures were morphed with one subset of the erotic female nude pictures.

When participants arrived at our laboratory, the experimenter asked them to sit in a comfortable chair approximately 80 cm in front of a computer screen with a visual angle of 25°. The experimenter attached electrodes for electromyographic (EMG) measurement (as described in Startle Pretest). Participants were instructed via computer screen that a series of

pictures would be displayed and that each picture should be viewed for its entire duration. Participants were also asked to relax, to neither move nor speak, and to avoid long periods of eye closure. Finally, they were told that brief noises would be delivered via headphones. Six startle probes presented before the experimental session served as habituation trials. Then the previously described pictures (self-resembling erotic female nudes, other resembling erotic female nudes, not manipulated erotic female nudes, and neutral pictures) were displayed on the computer screen in a randomized order for each participant. Each picture was shown for 5 s and an acoustic startle probe was presented between 2.5 and 3.5 s after picture onset in 8 (of 10) pictures per category, for a total of 40 pictures trials. A black screen was shown for 4 s in every Inter-Picture-Interval. The acoustic startle stimulus consisted of a binaurally presented burst of white noise (105 dB, 50 ms, instantaneous rise time).

Pleasure and Arousal Ratings. After completing the affective startle modulation paradigm the participants were asked to evaluate each picture for perceived pleasure and arousal using Self-Assessment-Manikin ratings ranging from 1 to 9 (1 indicates very low pleasure and arousal, and 9 very high pleasure and arousal) (Bradley & Lang, 1994).

After completing the affective startle modulation paradigm and the pleasure and arousal ratings participants filled out a small questionnaire about their opinion about the purpose of the experiment: They were asked if they found anything to be special about the pictures of the erotic female nudes. None of the participants correctly detected that the pictures were digitally altered. After completing the questionnaire the participants received 15 Euros and were thanked for their participation.

Debriefing. We did not inform participants about the true purpose of the experiment until we finished the data acquisition of all participants, because we wanted to avoid participants speaking about the experiment with other potential participants. After we finished data acquisition we contacted each participant and explained the purpose of the experiment. Participants were encouraged to contact the experimenter at any time if they had further questions.

2.2.5 Physiological recordings and data analysis

The startle response was assessed as peak EMG activity of the left orbicularis oculi, and was recorded with a BIOPAC MP 150 system and an EMG 100C amplifier at a sampling rate of 1000 Hz, with a notch filter of 50 Hz and a band pass filter of 28 to 500 Hz. The raw signal was rectified and integrated online with a time constant of 10 ms.

A semi-automated PC program was used to analyze EMG data. The algorithm identified response peaks in the time interval of 20-150 ms after stimulus onset and baseline

was set to 50 ms prior to stimulus onset. EMG data of all participants was manually confirmed with respect to non-response (no visible startle response) and/or artifacts (i.e. voluntary or spontaneous eyeblinks coinciding with the startle stimulus, trials with excessive background noise, multiple peaks).

The startle response was defined as the difference between a stable baseline (50 ms before stimulus onset) and the maximum magnitude of the EMG 20-150 ms after startle stimulus onset. If responses were not visible at the typical response latency of a particular participant response magnitude was set to zero. Zero response magnitude data were included in the averaging procedure, with startle response magnitude as the output measure. Raw data were T-scored using all blinks of each participant as the standard distribution in order to minimize between-participant variability in the absolute size of the startle response (Blumenthal, Elden, & Flaten, 2004).

A repeated measure analysis of variance (critical α level = .05) was used to analyze the effect of picture content (4 levels: self-resembling erotic female nudes, other-resembling erotic female nudes , not manipulated erotic female nudes, and neutral) on startle magnitude. Furthermore, a multivariate analyses of variance (critical α level = .05) with picture content as the within participant factor and pleasure and arousal ratings as dependent variables was conducted. Effect sizes are reported as partial eta-squared values.

2.3 Results

2.3.1 Startle Magnitude

To analyze the difference in sexual approach motivation to the different erotic picture categories, we had to first show that affective startle modulation actually took place. As expected, startle response magnitude was affected by picture content ($F(3, 87) = 15.989$, $p < .001$, $\eta^2 = .355$). Startle probes elicited larger blink responses during foreground presentation of neutral pictures compared to self-resembling erotic female nudes ($p < .001$), other-resembling erotic female nudes ($p < .01$), and not manipulated erotic female nudes ($p < .01$), showing the expected startle inhibition for appetitive pictures.

The experimental findings of central importance tested the hypothesis that the startle activity was different in the presence of pictures of self-resembling erotic female nudes compared to other-resembling erotic female nudes and not manipulated erotic female nudes. As expected, startle responses during foreground presentation of the three erotic photograph categories differed significantly from each other. Foreground presentation of pictures of self-resembling erotic female nudes led to a larger inhibition of the startle reflex compared to

other-resembling erotic female nudes (p <. 05) and not manipulated erotic female nudes (p <.05), indicating a higher sexual approach motivation to these pictures (Figure 2).
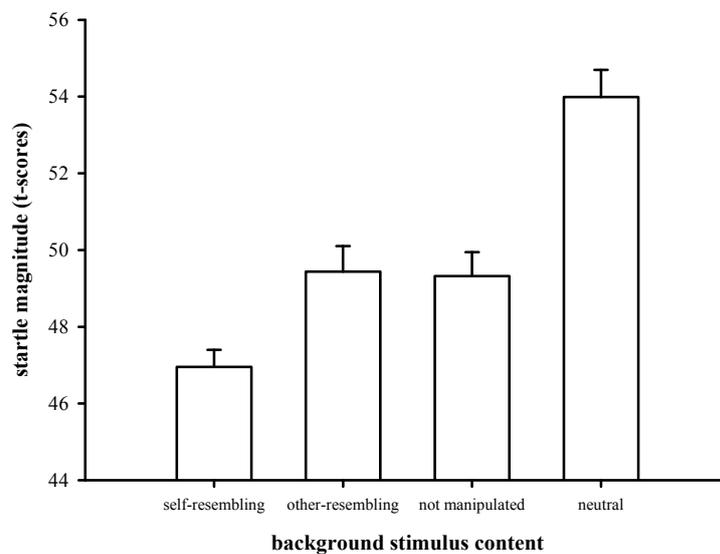
Figure 2:   Blink magnitude to startle stimuli during foreground presentation of four different picture contents (a) photographs of erotic female nudes with manipulated facial similarity to the participant (self-resembling), (b) photographs of erotic female nudes with manipulated facial similarity to another participant (other-resembling), (c) photographs of erotic female nudes whose facial characteristics were not manipulated (not manipulated), and (d) neutral pictures. Data are reported as T-Scores. Error bars indicate one standard error.

2.3.2 Self-report Pleasure and Arousal ratings

Analysis of picture valence ratings revealed a main effect of content $(F(3,87) = 47.02, p < .001, \eta^2 = .62)$. All three erotic picture categories were rated to be more pleasant than the neutral pictures $(p < .001)$. However, there was no difference in valence ratings between the three erotic categories. Ratings of pictures of self-resembling erotic female nudes did not differ significantly from that for pictures of other resembling erotic female nudes $(p = 1.00)$ or not manipulated erotic female nudes $(p = 1.00)$. Analysis of arousal ratings revealed a main effect in the same direction $(F(3,87) = 96.203, p < .001, \eta^2 = .77)$. All three erotic subsets were rated as more arousing than neutral pictures $(p < .001)$, but there was no significant difference in arousal ratings between self-resembling pictures of erotic female nudes and other-resembling pictures of erotic female nudes $(p = 1.00)$, and there was also no significant difference between ratings for self-resembling erotic pictures and not manipulated erotic pictures $(p = .298)$. These findings indicate that participants experienced all three erotic categories to be equally pleasant and arousing. Means and standard deviations are presented in Table 1.

Table 1: Mean reports of rated pleasure and arousal when viewing self-resembling erotic pictures, other-resembling erotic pictures, not manipulated erotic pictures, and neutral pictures.

Dependent measure	Self-resembling erotica		Other-resembling erotica		Not manipulated erotica		Neutral pictures	
	M	SD	M	SD	M	SD	M	SD
Pleasure ratings (1-9)	6.57 ± .73[a]		6.46 ± .65 [a]		6.75 ± .93 [a]		4.85 ± . 72	
Arousal ratings (1-9)	5.76 ± 1.29 [a]		5.70 ± 1.25 [a]		6.03 ± 1.43 [a]		2.64 ± 1.31	

Note. [a] Difference from neutral pictures is significant

2.4 Discussion

The purpose of the present study was to investigate whether facial self-resemblance has an influence on attractiveness ratings of pictures of erotic female nudes and sexual approach motivation to these pictures. In addition to subjective ratings, we used affective startle modulation to investigate the activity of neurobiological circuits involved in approach motivation. We predicted that facial self-resemblance should influence affective startle modulation to, and subjective ratings of, pictures of erotic female nudes. We showed that startle response magnitude was smaller during foreground presentation of photographs of self-resembling female nudes compared to other-resembling female nudes and not-manipulated female nudes. This indicates that facial self-resemblance increases the activity of neurobiological structures involved in approach motivation, suggesting that similar mates lead to a larger sexual approach motivation and, thus, to a larger sexual attractiveness than dissimilar mates. Although our results are based on a pre-attentive measure of approach motivation, the startle response, these results are in line with the findings that humans tend to prefer similar mates over dissimilar mates.

However, this effect was not found for subjective pleasure and arousal ratings, which did not differ from each other in the three erotic female nude picture categories. Thus, there is a dissociation between our two indicators of sexual attractiveness: Affective startle modulation and subjective ratings. This dissociation has been shown before (Lass-Hennemann, et al., 2009; Levenston, Patrick, Bradley, & Lang, 2000) and may be explained by the different processes underlying startle modulation and subjective ratings of picture content. As described above, subjective ratings are a voluntarily controllable measure, one that may be confounded by person variables such as social desirability and demand characteristics. Startle modulation, on the other hand, is not dependent on conscious

intentional control. Therefore, affective startle modulation might be a more valid measure of sexual approach motivation, especially in this specific context, since the problem of controllability of the answers that a participant gives might be even stronger in an erotic context, where factors like shame and embarrassment may play a role.

The dissociation between subjective ratings and affective startle modulation may also be explained by the opponent process theory of emotion (Solomon & Corbit, 1974a). According to this theory, emotional and motivational states are modulated by opposing reactions, the a and b processes. The a process is the initial reaction to an emotion-eliciting stimulus (in our case, the pictures of the erotic female nudes). The b process is a lagging compensatory reaction that tends to return the system to equilibrium, thereby modulating the affective experience. The affective valence of the b process is opposite to that of the a process. In our paradigm, affective startle modulation took place during picture viewing, whereas the subjective ratings were assessed at the end of the session, shortly after picture viewing. Therefore, the b process might already have been dominant during picture rating, explaining the absence of a difference in subjective ratings between self-resembling, other-resembling, and not-manipulated pictures.

One important methodological advantage of our study is that we employed a cross-over design, using the same erotic pictures for pairs of two participants, with the other-resembling erotic pictures for the one participant serving as the self-resembling pictures for the other participant. This indicates that the inhibition of startle reactions in the presence of the pictures of self-resembling erotic female nudes was based on the degree of self-resemblance with the participant, and not on some other characteristic of the pictures.

Two different processes could lead to the greater startle inhibition during pictures of self-resembling erotic female nudes compared to the other erotic picture categories. One explanation proposes an additive effect of the similar face and the attractive body. The similar face might be perceived as more attractive than the dissimilar faces and, therefore, enhances the affective valence of the erotic female nude by an additive effect of the similar, more attractive face, and the attractive body. Another explanation proposes that the similar face is more familiar to the participant and does not capture as much attention as the dissimilar faces. This could lead to a shift of attention from the face to the attractive body, leading to an enhanced startle inhibition. A replication of this study with a measure of eye-tracking would be an appropriate next step to test the hypothesis that facial similarity modulates the extent to which the face and attractive body of erotic female nude pictures are viewed. However, it must be remembered that, when we speak of perception, familiarity, and attention in these

explanations, the participants did not consciously realize that the self-resembling face actually included aspects of their own features. That is, the participants were not consciously aware of the manipulation of self-resemblance.

Our results are in line with the findings that human romantic partners tend to resemble each other, indicating a preference for similar mates (Bereczkei, et al., 2002; Bereczkei, et al., 2004). However, our results are not compatible with the findings of Debruine (2005), who showed that facial-self-resemblance decreased attractiveness in the context of a short-term relationship and had no effect on attractiveness in the context of a long-term relationship. However, Debruines' study and our study differ significantly from each other in several ways. For example, Debruine used a forced-choice paradigm in which participants viewed pairs of facial stimuli, which may have led to an artificially forced attractiveness decision which might not represent a participant's true opinion about the facial stimuli.

Another difference between our design and that of Debruine is that we used male participants only. We decided to use men because research has shown that men respond more to visual sexual stimuli than females (for a review see Rupp & Wallen, 2008). However, poor mating decisions may have less impact on men than they do on women, since men have a potential faster rate of reproduction and less costs of reproduction, and therefore tend to be less choosy in their mate choices (Geary, Vigil, & Byrd-Craven, 2004). Therefore, facial self-resemblance as a potential cue for inbreeding might be more relevant to women than to men, which might be tested by replicating the present study with women as participants. Future studies might also include other physiological measures, such as skin conductance and heart rate, to allow for a more detailed assessment of emotional engagement.

In spite of these limitations, our results are in line with many studies showing that romantic partners tend to resemble each other. The advantage of our study is that it is, to our knowledge, the first experimental manipulation of facial resemblance that yields this result. Furthermore our study is the first to use a sensitive, well-studied measure of sexual attractiveness (startle reactivity) and approach motivation that is not directly under the voluntarily control of the participant. Thus, our findings not only serve to further support facial similarity as a modulator of the attractiveness of, and sexual approach motivation to, potential mates, but it also shows that these effects can be found in the modulation of the startle reflex, a response that may represent a very basic measure of sexual approach motivation.

Chapter III: Effects of Cold Pressor Stress on the Human Startle Response

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3.0 Abstract

Both emotion and attention are known to influence the startle response. Stress influences emotion and attention, but the impact of stress on the human startle response remains unclear. We used an established physiological stressor, the Cold Pressor Test (CPT), to induce stress in a non-clinical human sample (24 student participants) in a within-subjects design. Autonomic (heart rate and skin conductance) and somatic (eye blink) responses to acoustic startle probes were measured during a pre-stress baseline, during a three minutes stress intervention, and during the subsequent recovery period. Startle skin conductance and heart rate responses were facilitated during stress. Compared to baseline, startle eye blink responses were not affected during the intervention but were diminished afterwards. These data describe a new and unique startle response pattern during stress: facilitation of autonomic stress responses but no such facilitation of somatic startle eye blink responses. The absence of an effect of stress on startle eye blink responsiveness may illustrate the importance of guaranteeing uninterrupted visual input during periods of stress.

Keywords: stress, startle, eye blink, heart rate, skin conductance, electro-dermal response

3.1 Introduction

Stress is regarded to be an adaptive reaction to an adverse stimulus or situation. The stress response is a multi-level, complex shift in the organism's physiological and psychological functioning (Del Giudice, et al., 2010). The physiological stress response allocates bodily resources to facilitate quick, evasive actions at the expense of more long term, regenerative functions. Acute stress involves an endocrinal response (de Kloet, 2003) and activation of the sympathetic nervous system (Ulrich-Lai & Herman, 2009), and influences somatic motor behaviour and psychological adjustments.

In contrast to the low-level, biological adaptations, that meet the change in energy demands, stress effects on basic psychological processes, and interaction with attention and emotion, are less well understood. Previous studies found an attentional bias for aversive, threatening stimuli under stress exposure (Chen, et al., 1996; Mogg, et al., 1990), while others found no (McNally, et al., 1992; Mogg, et al., 1993) or even opposing effects, with less attention for negative stimuli after stress manipulation (Ellenbogen, et al., 2002; Mathews & Sebastian, 1993). However, differences of independent and dependent variables used in the above cited studies complicate the search of answers. The experimental paradigm of startle eye blink modification may provide a biology-based measure of emotional and attentional effects that might clarify these questions.

The startle response is a fast defensive mechanism that protects the organism against potential injury. Elicited by abrupt and intense stimuli in various sensory modalities, the startle response protects the organism against imminent physical harm in a natural setting, e.g. due to a predator or a blow (Koch, 1999). Somatic muscle contractions and activation of the autonomic nervous system (ANS) represent the two major components of the response. Sudden contractions of facial and flexor skeletal muscles induce a defensive posture and protect essential parts of the body. Acceleration of heart rate and increased skin conductance responses (SCR) indicate an activation of the ANS and prepare the organism for action, e.g. fight or flight (Davis, 2002).

The emotional context in which startle is elicited may modulate the response magnitude in one of two directions. Positive emotional states attenuate and negative states enhance the startle response. Such affective startle modulation has been explained in terms of motivational priming: aversive emotional stimuli prime the defensive motivational system and thereby facilitate defensive reflexes, whereas appetitive emotional stimuli inhibit defensive reflexes (Lang, et al., 1990). Experimental paradigms have employed a diverse array of

emotional stimuli with positive or negative hedonic valence, such as pictures (Bradley, et al., 1990), films (Kaviani, et al., 1999), music (Roy, et al., 2009), odours (Pause, et al., 2009) or anxiety-inducing darkness (Muhlberger, Wieser, & Pauli, 2008). Also, placebo effects of neutral stimuli (Flaten, Aasli, & Blumenthal, 2003) and the imagination (McTeague et al., 2009) or anticipation (Sabatinelli, Bradley, & Lang, 2001) of emotional content modulate the startle response.

However, although it is typical that psychological ratings indicate a negatively valenced emotional stress response, e.g. increased ratings of adversity, irritability, anxiety, and loss of control, the effect of stress on startle responsiveness remains unclear. A prototypical laboratory stressor is the well defined Cold Pressor Test (CPT) (Hines, 1940; Schwabe, Haddad, & Schachinger, 2008). De Peuter et al. (2009) found potentiated startle responses during a one minute CPT. While this result is consistent with motivational priming, other studies found opposing effects. Tavernor et al. (2000) used a 90 s CPT and found lower startle magnitudes in the CPT condition. However, the ice water hand immersion during the CPT was rather brief in both studies, as compared to earlier studies in which immersion lasted up to 6 min. (Halter, Stratton, & Pfeifer, 1984; Stratton, Halter, Hallstrom, Caldwell, & Ritchie, 1983). With such a time schedule, only three startle noise presentations, a very limited number in human startle research, were delivered during the CPT in the Tavernor et al. study. Considering that CPT stress effects may need some time to develop, e.g. the first 30 s of ice water hand immersion are often well tolerated, and strongest blood pressure increases appear during the second minute (Fagius, Karhuvaara, & Sundlof, 1989; Stancak, Yamamotova, Kulls, & Sekyra, 1996; Victor, Leimbach, Seals, Wallin, & Mark, 1987; Yamamoto, Iwase, & Mano, 1992), it may be speculated that not all startle probes were delivered during a genuine stress experience of the participants, thus making the time course of effects incomparable to De Peuter's study. In the current study we aimed to investigate the effects of a longer lasting (3 min) CPT version on the human startle response, with special focus on the different startle response components, e.g. somatic motor vs. autonomic responses.

Somatic motor reactions occur faster than changes of the autonomic nervous system (ANS). Motor startle reactions have been shown in various human muscle systems such as facial (Berg & Balaban, 1999), cervical, or limb muscles (Brown, et al., 1991). Indicators of the autonomic startle response are cardiovascular (Holand, et al., 1999; Richter, Deter, et al., 2011) and SCR changes (Vrana, 1995). Interestingly, the magnitudes of these response components do not always show common variation. Such response separation is shown, for

example, by the fact that startle eye blink responses exhibit differences in habituation from SCR (Bradley, 2009; Bradley, Lang, & Cuthbert, 1993) and cardiovascular startle responses (Mata, Rodriguez-Ruiz, Ruiz-Padial, Turpin, & Vila, 2009). So far, it is unclear whether stress affects startle ANS and somatic motor responses in a similar way, or whether it may induce a response separation.

Acute stress effects may carry over into the recovery period. Since we wanted to compare pre, during, and post stress effects, we decided to use equally long periods (3 min) with an equal number of startle probes before (pre), during and after (post) the CPT intervention. We measured autonomic startle responses in heart rate and SCR. The eye blink response was measured by recording the electromyographic (EMG) response of the orbicularis oculi muscle, and we also measured the actual eyelid movement via video recordings. This method allowed us to study the kinematics of the startle eye lid movement, which is essential to identify the consequences of the startle eye blink response for the continuation of the visual signal input flow.

3.2 Methods

3.2.1 Participants

Twenty-four undergraduate students of the University of Trier participated in this study. Participants were interviewed for actual and past medical and/or psychiatric health problems. Resting blood pressure (BP) (Dinamap System, Critikon, US) was assessed. Exclusion criteria were acute or persistent medical and psychiatric diseases, current medication except the occasional use of pain killers (paracetamol, aspirin, or NSAR), actual or past hearing problems (e.g. tinnitus), a history of fainting, and BP greater than 140/90 mmHg or systolic BP lower than 110 mmHg.

Four participants were excluded from further analysis because of complete loss of startle eye blink responsiveness during the initial habituation phase ("nonresponder"). The final sample included 20 participants (11 f/ 9m, mean age = 24.29 y, SD = 2.53 y).

Participants gave a written informed consent and were financially compensated with 15€ for participation. Experimental procedures were approved by the ethical committee of the medical association of Rhineland-Palatinate.

3.2.2 Experimental Design

Each participant was subjected to both conditions in two separate blocks: stress (Cold Pressor Test, CPT) and control intervention (hand immersion in warm water), with a resting break of 45 minutes in between the two blocks. The sequence of conditions was counterbalanced between subjects.

Each block was divided into three phases: pre-intervention (phase 1; 4 min), intervention (phase 2; 3 min), and post-intervention (phase 3; 3 min). In phase 1, 12 startle probes were presented, in each of the other phases 8 startle probes were presented (see Figure 3)
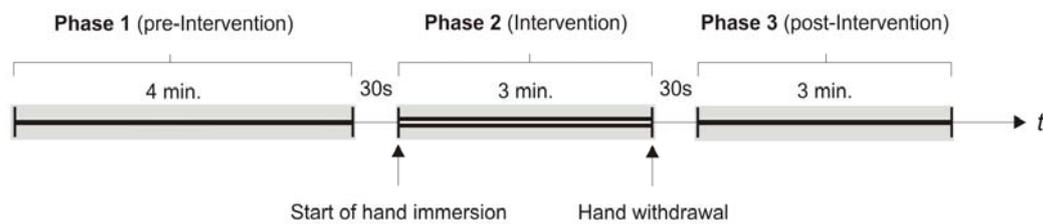
Figure 3: Experimental protocol.

3.2.3 Stress Intervention

The CPT was used to elicit a physiological stress response. The participants' right hand was placed in a bucket with crushed ice and water up to the wrist. Water temperature ranged between 0° and 4°C. Participants were instructed to leave the hand in the water for a period of three minutes.

The control condition was structured identically to the stress condition, with the only difference being that the water temperature was near body level (35° C).

3.2.4 Startle Stimulation

Startle stimuli were acoustic white noise probes (105 dB, 50 ms duration, instantaneous rise time, binaural stimulation) presented via audiometric headphones (Holmco PD-81, Holmberg GmbH & Co. KG, Germany). Startle probes were presented with a variable inter-stimulus-interval of 10 to 16 s. The first four startle probes during phase 1 served as habituation trials and were not included in further analysis[1].

3.2.5 Procedure

Experiments were performed in the afternoon between 2 and 6 p.m. After being checked for exclusion criteria and signing their consent form, participants were familiarized with the laboratory setting. They were seated in front of an eye tracker (SMI iView-X HiSpeed 500), mounted on a height-adjustable table, with a 19" - TFT-monitor (1280 x 800 resolution). The distance between eye tracker and monitor was 35cm. The participant was instructed to adjust to a comfortable sitting position. Electrodes were attached and the participant placed the head in a stable position, with forehead and chin resting on the eye tracking device throughout the experiment. The eye tracker was calibrated to control for the participant's gaze position and headphones were adjusted.

The experiment started with an instruction appearing on the screen, informing the participant that the experiment would start and to sit quietly, keep their eyes in the direction of a fixation cross that would appear in the middle of the screen, and neither move nor speak. The experiment would start with the grey fixation cross appearing on a black screen. This cross remained on the screen throughout the experiment.

Subsequent to phase 1, the experimenter took the participant's hand and placed it in the water bucket. After three minutes, the experimenter removed the hand, dried it with a towel and placed it on the table again. The participants gaze remained on the screen throughout the experimental intervention. Phase 3 started one minute after the end of the water immersion.

3.2.6 Data acquisition and Analysis

3.2.6.1 Electromyography

Electrodes for EMG recording of the m. orbicularis oculi were attached below the participant's right eye at an inter-electrode distance of 1.5 cm. The EMG-signal was recorded on hard disk with a BIOPAC MP 150 system and an EMG 100C amplifier via Tyco Healthcare H124SG electrodes at 16 bit resolution and 1 kHz sampling rate. Hardware band-pass filter settings were 10 to 500 Hz, followed by a 28 Hz software high-pass filter (van Boxtel, Boelhouwer, & Bos, 1998). The raw signal was rectified and integrated online with a time constant of 10 ms (Blumenthal, 1994).

The EMG-startle responses were analyzed offline with a C++ based, semi-automated program. Startle response was defined as difference between peak and baseline signal. The integrated algorithm identified peak in a time interval between 20-150 ms after stimulus onset. Baseline was assessed 50 ms prior to stimulus onset (Lass-Hennemann et al., 2010).

Each response was manually confirmed and corrected for non-responses and artefacts. Non-responses (cases with no discernable response) were set to zero and included in the analysis (1.09 % of all responses). Cases with electrical and physiological artefacts (such as voluntary or spontaneous eye blinks coinciding with the startle stimulus, trials with excessive background noise or multiple peaks) were excluded from analysis (2.83 % of all responses). Responses were averaged across participants for each condition. Zero response data were included in the averaging procedure, with startle response magnitude as the final output measure (Blumenthal, et al., 2005).

3.2.6.2 Image Eye Blink Recording

The motion of the left eyelid was assessed with an image based approach.[2] The start of image recordings was synchronized with the onset of the acoustic startle probe. Each recording sequence lasted 600ms. The images were recorded at a frequency of 500Hz, thereby generating 300 images per trial. All images were of 256 bit depth greyscale resolution; image size was 224x160px. Pictures were assessed with the internal eye camera of the eye tracker and a customized version of the iView recording software (SMI iView 2.5, SensoMotoric Instruments GmbH, Teltow, Germany). The images were automatically saved to the hard disk of the eye tracking computer.

Image analysis was conducted manually by measuring the distance between upper and lower eyelid on the mid pupil position in the picture at startle probe onset and in the picture with the maximum eye closure. Lid distance was measured in pixels with a digital ruler (Pixel Ruler 4.0, Mioplanet, Rimouski, Canada). Maximal eye closure was expressed as the percentage of eye lid closure at the point of maximal closure in relation to baseline lid distance at the beginning of the trial. Responses were averaged across participants for each phase.

In addition to being the baseline for blink quantification, initial lid distance (aperture) is reported as a measure of muscle tone of the upper eyelid.

3.2.6.3 Cardiovascular Data

Electrodes for ECG-measurement (ECG Tyco Healthcare H34SG Ag/AgCl electrodes, diameter: 45 mm) were placed according to a standard lead II configuration. The signal was acquired with the BIOPAC MP 150 and a ECG100 Amplifier. The signal was high-pass filtered (0.5 Hz, hardware filter) and stored to disk (1 kHz). Beat-to-beat heart rate data were

calculated by a semi-automatic QRS detection in WinCPRS (Absolute Aliens Oy, Turku, Finland). Responses were averaged across participants for each condition.

Heart rate was calculated in beats per minute (bpm). We analysed the mean heart rate values for each condition as well as the startle heart rate response, defined as the change between the time period 4 to 6s post-startle and the -2 to 0 s pre-startle baseline before startle stimulus presentation.

3.2.6.4 Skin Conductance Responses (SCR)

Skin conductance was measured with BIOPAC MP 150 and a GSR100 Amplifier. Electrodes were the same as for ECG. Electrodes were placed on the palm of the left hand, the signal filtered with a 10 Hz low pass filter.

For SCR analysis we employed the same program that we used for EMG analysis. The response was defined as the peak in a time period of 4-6s post startle stimulus. Baseline was measured as the mean in the period 2 s before the startle probe. All individual SCRs were log-transformed, and then normalized [Z(log (1+SCR)] per participant. Averaging was done per phase, condition, and participant.

3.2.6.5 Subjective Ratings

After the experiment was finished, participants were asked to rate the degree of unpleasantness of the experimental manipulation on a Likert scale ranging from 0 ('not at all unpleasant') to 8 ('very unpleasant').

3.2.7 Statistical Analysis

EMG response, eye lid response, skin conductance response, heart rate response, and mean heart rate were analysed in a repeated measures 3×2 ANOVA, with the factors 'time' (phase 1: pre-intervention, phase 2: intervention, phase 3: post-intervention) and 'treatment' (CPT vs. control) for each dependent variable.

The interaction term is reported, as well as 'a priori' defined contrasts reflecting the intervention effects. Contrasts were constructed between phase 1 and phase 2 for CPT vs. the control condition. This contrast reflects the cold pressor stress effect. In a similar way, the post-stressor effects were analysed: contrasts between phase 1 and phase 3 for CPT vs. the control condition. P-values for factors with more than two conditions are reported after Greenhouse–Geisser correction.

Subjective ratings of unpleasantness were analysed for an effect of the level of stressor with a Student's *t*-test for paired samples.

The critical alpha-level was set to .05 in all analyses.

3.3 Results

3.3.1 Subjective Ratings

The CPT was rated as significantly more unpleasant than the control condition (t_{18} = 15.48; p<.001, see Table 2).

3.3.2 Mean Heart Rate

During the CPT intervention, we found an increase in mean heart rate, which was not present in the control condition. The ANOVA revealed a significant overall interaction between "treatment" (CPT, control intervention) X × "time" (pre, during, post intervention) on mean HR ($F_{2,38}$ = 17.58; p<.001; η^2=.48), contrasting HR from phases 1 (pre) and 2 (during) over the intervention blocks, revealed increasing HR during the CPT ($F_{1,19}$ = 13.63; p<.01; η^2=.42). There were no statistically significant effects with regard to pre-post intervention differences (see Table 2).

3.3.3 Initial Eyelid Distance

Compared to the control condition, we found an increased initial eyelid distance during the intervention in the CPT condition. The contrast between phases 1 (pre) and 2 (during) over the intervention blocks, revealed increased initial eyelid distance during the CPT ($F_{1,19}$ = 5.18; p<.05; η^2=.21) (see Table 2).

3.3.4 Startle EMG Response

Startle EMG magnitude was significantly reduced during intervention ($F_{1,19}$ = 8.85; p<.001; η^2=.89). The ANOVA revealed a significant overall interaction between "treatment" (CPT, control intervention) × "time" (pre, during, post intervention) on startle EMG responses ($F_{2,38}$ = 4.54; p<.05; η^2=.11). There were no statistically significant differences nor interactions considering the kind of treatment (CPT vs. control) during the intervention (phase 2).

However, after the intervention, we found lower response magnitudes in the CPT condition then in the control condition. This is expressed in the contrast between phases 1 (pre) and 3 (post) over the intervention blocks ($F_{1,19}$ = 7.13; p<.05; η^2=.19) (see Figure 4A).

3.3.5 Startle Eyelid Response

No significant main effects, nor an interaction between "treatment" (CPT, control intervention) X "time" (pre, during, post intervention) was found for the startle eyelid responses (see Table 1).

Table2: *Effects of treatment on physiological and subjective parameters: Mean (SD)*

	CPT			Control		
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3
Eye Lid Distance - blink (nadir during blink)	46.81 (29.9)	38.99 (32.2)	39.14 (27.49)	47.24 (27.07)	36.88 (29.26)	41.3 (29.84)
Eye Lid Distance - baseline (initial aperture)	50.7 (6.92)	53.09 (6.02)	50.55 (7.2)	52.09 (5.32)	49.17 (6.04)	48.57 (6.76)
Heart Rate	68.36 (12.02)	75.95 (14.77)	67.59 (11.87)	68.76 (10.61)	69.27 (11.16)	69.16 (10.32)
Probability of Complete Blink	21 (34)	19 (33)	14 (25)	18 (33)	15 (31)	17 (36)
Subjective Ratings for Unpleasantness		6.37 (1.30)			0.89 (0.87)	

Eye Lid Distance - blink: distance covered as percent of baseline distance; *Eye Lid Distance - baseline*: pixel; *Heart Rate*: bpm;
Probability of Complete Blink: percent; *Subjective Ratings*: 8-digit Likert scale.
doi:10.1371/journal.pone.0049866.t001

3.3.6 Startle Skin Conductance Response

We found a significant overall interaction between "treatment" × "time" ($F_{2,38}$ = 4.81; p<.05; η^2=.20). Startle SC responses increased during the intervention in the CPT condition, while no such effect was found for the control condition, as expressed in the contrast from phases 1 (pre) and 2 (during) over the intervention blocks ($F_{1,19}$ = 6.19; p<.05; η^2=.25). There were no statistically significant effects with regard to pre-post intervention differences (see Figure 4 B).

3.3.7 Startle Heart Rate Response

We found a significant overall interaction between "treatment" × "time" on startle HR responses ($F_{2,38}$ = 4.98; p<.05; η^2=.21). Startle Heart Rate Responses increased during the intervention in the CPT condition, while no such effect was found for the control condition, as

expressed in the contrast from phases 1 (pre) and 2 (during) over the intervention blocks ($F_{1,19}$ = 5.41; p<.05; η^2=.22). There were no statistically significant effects with regard to pre-post intervention differences (see Figure 4 C).
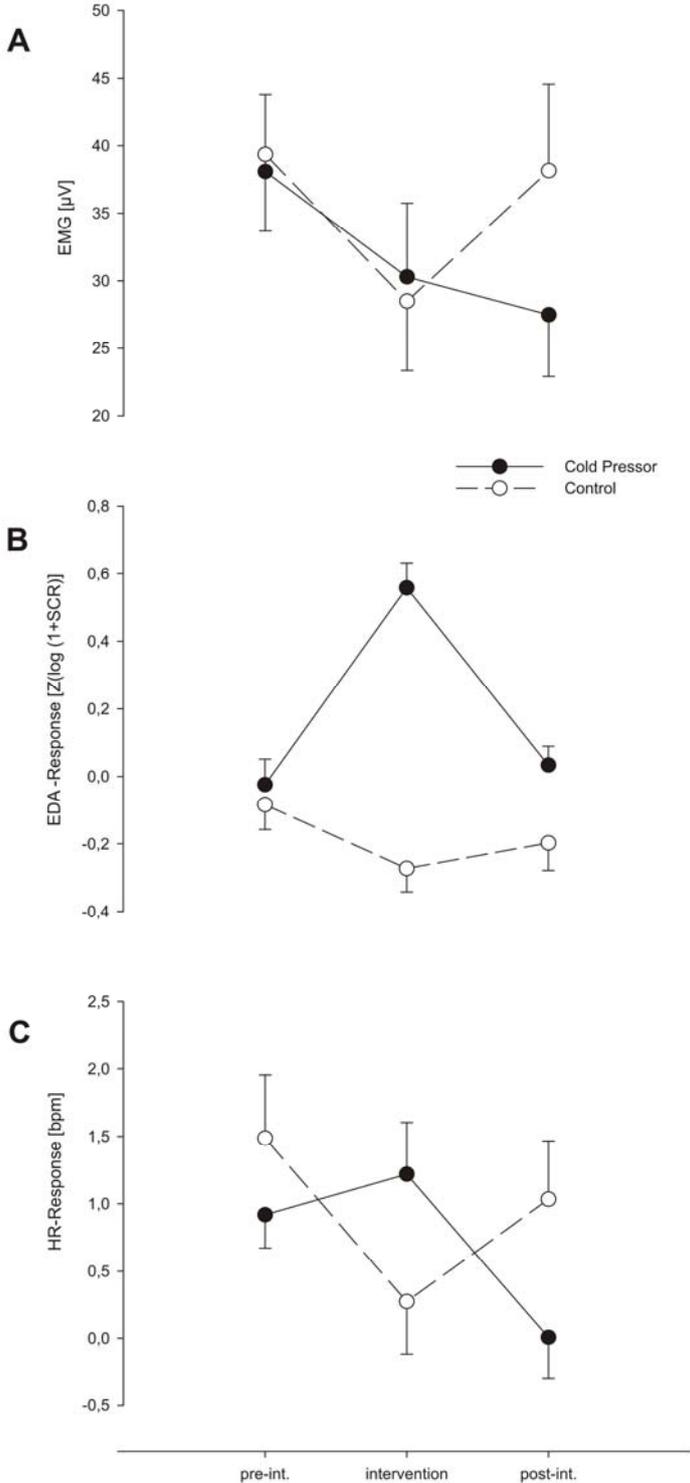

Figure 4:   Measures of the startle response: eye blink (EMG)-, skin conductance and heart rate responses.

3.4 Discussion

In this study, we investigated the influence of cold pressor stress on somatic motor and autonomic components of the human startle response. We identified a unique stress modulation pattern of startle response components, not seen before during experimental manipulation of emotion or attention. The pattern consists of selectively enhanced autonomic startle reactivity, as would be expected during aversive emotional states, but not somatic motor eye blink responsiveness. As such, stress supports individual adjustments to startling danger sources by boosting ANS effects (o.g. on energy supply), but avoiding excessive eye blinks which compromise the continuity of visual input signals.

The CPT intervention of the current study proved to be successful; our participants rated the CPT intervention as significantly more unpleasant than the control intervention, thereby confirming the subjective aversive component of the stress intervention. Mean heart rate was significantly elevated during the CPT intervention and returned to baseline afterwards, indicative of a sustained autonomic activation during the CPT. This is in line with previous findings (e.g.Saab et al., 1993) and can be attributed to increased cardiac sympathetic activity (Victor, et al., 1987).

Startle-evoked autonomic responses were significantly affected by the stress intervention. Startle skin conductance responses were increased during the CPT. Such responses are indicative of higher sympathetic activation and could be expected based on previous studies (e.g.Vrana, 1995). During the stress intervention a similar pattern of enhanced heart rate startle response was found. However, heart rate responses were lower in the recovery period after the stress intervention. The lowered responsiveness after the CPT could be interpreted as a counter regulatory mechanism: heart rate might decrease as a result of increased vagal outflow, which is mediated by enhanced baroreflex sensitivity in response to sustained blood pressure increases (Mourot, Bouhaddi, & Regnard, 2009).

The CPT had a different effect on startle-evoked somatic motor responses. The CPT manipulation did not reveal a significant difference for EMG-measured orbicularis oculi muscle activity during the intervention. Compared to the pre-intervention baseline, startle magnitude was significantly reduced in both conditions, Subsequent to the intervention, post-stress startle magnitude was significantly lower compared to baseline in the CPT condition. This post-stress effect is comparable to the above described startle heart rate response. However, the EMG response pattern of m. orbicularis oculi activity did not translate into actual kinematic eyelid response. For this measure, no differences were found.

The EMG response pattern demands further explanation. In the presence of differential autonomic responses during the CPT intervention, we found no difference in the eye blink response during the CPT. Motivational priming would predict a potentiation of eye blink startle in an unpleasant state. However, it may be the case that different aspects of the intervention had a differential influence on the startle response. Attentional processes could possibly counteract affective modulation. Considering the more intense stimulation in the cold pressor condition, as compared to the control condition, we would expect that more attentional resources are directed to the stressor. This would imply that more attention is channelled to thermoceptive and nociceptive input, making attention less available for auditory processing. Directing attention towards the startle eliciting modality can increase startle, whereas directing it to a different modality can reduce startle magnitude (Filion, et al., 1998). Attentional and emotional factors interact and may work in opposite directions, making the net effect on startle responsiveness difficult to predict (Haerich, 1994). If they are equal in size and point in opposite directions, no observable net effect would appear. This may have been the case for the startle eye blink EMG response. The affect-related increase and the decrease due to attentional focussing would cancel each other out, ultimately provoking the same response magnitudes as in the control condition.

We found lower EMG eye blink responses in the recovery phase, after the intervention was terminated. With the cessation of the experimental manipulation after phase 2, attentional capture can be ruled out as a possible explanation while affective factors still have an impact. In fact, this pattern is in line with the motivational opponent-process theory (Solomon & Corbit, 1974b): during the recovery from an emotional stimulation, valence is predicted to reverse. The cold pressor test has a strong negative valence due to its subjective painfulness, which is known to increase during the time course (Streff, Kuehl, Michaux, & Anton, 2010). Relief itself is highly pleasant and rewarding (Andreatta, Muhlberger, Yarali, Gerber, & Pauli, 2010; Leknes, Brooks, Wiech, & Tracey, 2008). This offers an explanation for the attenuated EMG startle response after relief from the unpleasant stressor. The result also corresponds to a study by Franklin et al. (2010), that employed the CPT (120s) as a proxy for non-suicidal self-injury. Startle response magnitude was taken as a measure for cognitive–affective regulation (with pre-pulse inhibition reflecting the cognitive component). This study focused specifically on the pain component of the CPT, actually treating the CPT as a way to gain relief from a previous social-stress intervention. Franklin et al. also found reduced startle after the CPT. However, startle responses were measured only after, but not during, the CPT intervention in this study.

Furthermore, the kinematic analysis of eye lid movements did not reveal a stress effect. This is interesting, since video-measured eye lid movements shows a high correlation to the EMG (Deuter, Kress, Gemmar, & Schachinger, 2010) and startle evoked eye lid closure was susceptible to other experimental manipulations, such as affective startle modulation (Anders, Weiskopf, Lule, & Birbaumer, 2004) or prepulse inhibition (Lovelace, Elmore, & Filion, 2006). We also controlled for the probability of complete blinks (when the eyeball is fully covered by the lid), since these cases can diminish the correlation between lid movement and EMG. However, the probability was low and did not vary between phases or conditions. One possible reason for the absence of an effect in this measure is the impact of muscles other than the orbicularis oculi .The upper eyelid movement is accomplished by the interplay of two skeletal muscles, the orbicularis oculi muscle and the levator palpebrae muscle, as well as the smooth Müller's muscle, that runs from the musculus levator palpebrae to the upper margin of the tarsal plate. While a blink is basically accomplished by rapid activation of the orbicularis oculi muscle, the other two play a crucial role in lid elevation and upper eyelid tone (Bang et al., 1998; Evinger, Manning, & Sibony, 1991). Since Müller's muscle is sympathetically innervated (Schmidtke & Buttner-Ennever, 1992), stress (e.g. as in the CPT) could possibly influence the upper eyelid's muscle tonus and thereby also the movement during the blink. Indeed, we could demonstrate that the initial lid distance (measured as the aperture at the beginning of each trial) was increased during the CPT intervention, which was not the case in the control condition. This would support the hypothesis that stressful situations require a continuation of visual input to conquer potential threats.

The decrease in magnitude after the intervention could only be found in the EMG eye blink measure, not for the startle ANS responses. We are not able to explain the mechanism underlying such a response discrepancy. However, depending on the situational context, separate response components are weighted differently. Acute stress induces a large scale shift in attentional processing, with increased alertness and activation in defence-related processing structures (Hermans et al., 2011). Even though the startle related lid closure is adaptive by protecting the sensitive eye in the face of danger, it has detrimental effects as well. For a brief, but potentially crucial moment, visual input is interrupted. Still, guaranteeing continuous visual input during periods of stress offers some adaptive potential, since it may allow for more rapid and directed defence and escape behaviour. This result would also be in line with a recent study that found a dissociation between autonomic and electrophysiological responses to a CPT, indicating regulatory processes that preserve sensory perception (Woods et al., 2011). Therefore, the blink magnitude might reflect a

compromise between the need for protecting this vital organ on the one hand and not hindering appropriate action on the other hand.

Some limitations of this study need to be addressed. The validity of the results might be restricted to CPT-induced stress. The CPT distinguishes itself from other interventions in that it is a representative autonomic stressor with a specific activation of the sympathetic nervous system (Cui, Wilson, & Crandall, 2002; Lass-Hennemann, et al., 2010; Mourot, et al., 2009; Schulz, Plein, Richter, Blumenthal, & Schachinger, 2010). An additional reason to choose this intervention was the feasibility of video based blink recording without the participant being visually distracted. Still, how these results generalize to other stressful interventions remains open to further research. The degree to which the post-stress effects are mediated by humoral factors could not be addressed in this study. For that purpose, extending this paradigm over a longer post-stress time period would be of interest.

In conclusion, we have demonstrated that cold pressor stress has an effect on the acoustic startle response in humans. However, different components (somatic eye blinks, ANS responses) of the response are differentially affected. The resulting unique pattern of responses would allow for the benefits of ANS adjustments, but still guarantee the continuous input of visual signals.

Chapter IV: Startle effects on saccadic responses to emotional target stimuli

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4.0 Abstract

Startle stimuli elicit various physiological and cognitive responses. This study investigated whether acoustic startle stimuli affect saccadic reactions in an emotional pro- or anti-saccade task. Startle probes were presented either 500ms before or simultaneous with an imperative stimulus that indicated whether a saccade towards or away from positive, neutral, or negative peripheral target pictures had to be performed. Valence interacted with saccade direction according to an approach-avoidance pattern of gaze behavior, with delayed pro-saccades to negative targets and anti-saccades away from positive targets. Acoustic startle stimuli preceding the presentation of peripheral target pictures speeded up the initiation saccades, irrespective of stimulus valence. Results indicate a speeding of cognitive-motor processing by preceding startle stimuli.

Keywords: Pro-saccade, Anti-saccade, Affective Startle Modulation, Approach-Avoidance, StartReact, Electromyography, Prepulse Inhibition, Accessory Stimulus

4.1 Introduction

The startle reflex is a defensive response that is found in many species. It protects the organism against potential injury and prepares for adaptive action (Koch, 1999). Some of the most prominent startle response components in mammals involve the immediate contraction of facial and skeletal muscles. This movement pattern induces a protective posture and shields sensitive organs, such as the eyes, against traumatic impact. Beyond that, startle stimuli may enhance reaction times (Valls-Sole, et al., 2008), and interrupt ongoing cognitive processes in order to focus attention towards the source of the startle stimulus (Graham, 1979; Herbert, et al., 2006). Startle stimuli elicit P300, event related EEG-potentials, indicating that startle triggers the engagement of attention (Schupp et al., 2004; Schupp, Cuthbert, Bradley, Birbaumer, & Lang, 1997). Attending to and identifying danger sources allows for specific defensive action, thereby enhancing the chance to avoid injury. This is what would be expected if an aversive startle stimulus primed defensive cognitive processing. However, it remains to be demonstrated that startle stimuli indeed drive attention specifically towards aversive, harmful, and threatening stimuli. Alternatively, startle may affect the cognitive processing of different sorts of stimuli, aversive and appetitive, in a similar, unspecific fashion.

Emotional stimuli reveal important information: Depending on the valence, they are considered either beneficial or harmful to the organism. Since they provide an indicator of biological significance, emotional stimuli engage attention more than neutral stimuli (Mayer & Merckelbach, 1999). Spatial orienting to those relevant stimuli is facilitated; emotional stimuli are detected faster in a visual array (Ohman, Lundqvist, & Esteves, 2001). This effect can be demonstrated with various behavioral paradigms such as the emotional Stroop task (Dresler, Meriau, Heekeren, & van der Meer, 2009), the dot probe task (Koster, Crombez, Verschuere, & De Houwer, 2004; Miyazawa & Iwasaki, 2009) or the go/ no-go task (De Houwer & Tibboel, 2010). However, while mainly negative, threatening stimuli were initially considered to be preferentially processed (Ito, Larsen, Smith, & Cacioppo, 1998) recent studies suggest that the effect is based more on stimulus arousal than valence (de Oca, Villa, Cervantes, & Welbourne, 2012; McConnell & Shore, 2011; Most, Smith, Cooter, Levy, & Zald, 2007; Sheth & Pham, 2008; Vogt, De Houwer, Koster, Van Damme, & Crombez, 2008). Additionally, several state and trait variables exert an influence on this attentional engagement. Highly anxious individuals are more strongly biased to process negative stimuli (Van Honk, Tuiten, de Haan, vann de Hout, & Stam, 2001; Wilkowski, Robinson, Gordon, &

Troop-Gordon, 2007), an effect that has also been described for other personality variables (Amin, Todd Constable, & Canli, 2004) and psychopathologies such as dysphoria (Sears, Thomas, LeHuquet, & Johnson, 2010) or depression (Sears, Newman, Ference, & Thomas, 2011).

State factors have a moderating effect on attentional biases: Higher state anxiety has been found to increase interference effects in an emotional Stroop paradigm (Dresler, et al., 2009). Arousal, either evoked by the stimulus itself or by a different sources, can increase the competitive advantage of emotional stimuli (for a review, see: Mather & Sutherland, 2011). For example, stress could increase the impact of emotional distracters in a working memory task (Oei et al., 2012). Finally, motivational orientation, i.e. approach or avoidance, can influence how attention is captured by emotional distracters (Memmert & Cañal-Bruland, 2009; Rothermund, Voss, & Wentura, 2008).

The current study evaluated the impact of approach and avoidance on cognitive processing by employing eye tracking methodology. Eye movements provide a valid measure of early orienting because the gaze is shifted to the attended location (Henderson, 2003). In a recent study that compared a manual versus saccadic response task, saccadic eye movements were more strongly influenced by target valence in a forced-choice reaction paradigm: Emotional and neutral faces were presented in pairs, and participants had to perform either button presses or saccades towards the emotional or neutral target stimulus (Bannerman, et al., 2009). When emotional and neutral pictures are presented together in peripheral vision, the emotional picture is more likely to be fixated first and for a longer time, even if the task explicitly demands that the emotional picture is to be ignored (Nummenmaa, et al., 2006). In a pro- and anti-saccade task that required the participant to perform voluntary saccades toward or away from an emotional target stimulus, pro-saccades were faster towards pleasant stimuli when presented in the right hemifield (Kissler & Keil, 2008). This task required the participant to initiate voluntary saccades towards a lateral, peripherally presented stimulus (pro-saccade), or away from it (anti-saccade), depending on an imperative stimulus at central fixation. Anti-saccades induce a conflict situation between endogeneous and exogeneous attention: The reflexive tendency to look at the stimulus needs to be inhibited in favor of a controlled saccade in the opposite direction. While the emotionality of stimuli clearly has an influence on gaze patterns and saccades, the results regarding specific valence of the targets are less clear. Several studies have found enhanced processing of positive and negative stimuli (Calvo, et al., 2008; Nummenmaa, et al., 2006, 2009), while others have found

facilitating effects only for negative stimuli (Gutiérrez & Calvo, 2011; Lundqvist & Öhman, 2005).

In our study, participants had to perform pro- or anti-saccades to emotional, parafoveally presented target pictures. At the beginning of each trial, the gaze was directed to a fixation cross in the center of the screen. An emotional or neutral picture would appear in the left or right periphery of the screen. With a stimulus onset asynchrony (SOA) of 200ms, the fixation cross changed color to indicate which type of saccade was to be performed (imperative stimulus). Participants were instructed to keep their gaze at the fixation cross until the imperative stimulus appeared, i.e. reflexive saccades towards the pictures had to be inhibited. During this time period, a processing of the "emotional gist" of the target images should have been achievable (Phillips, 2009).

Two startle conditions were included in the experiment, early and late. In the early condition, acoustic startle probes were presented before the target images appeared. The startle stimuli in this condition could exert an influence on the processing of the image content at an early stage. In the second startle condition, startle stimuli were presented simultaneous with the imperative stimulus. Startle in this condition would primarily affect premotor and motoric processes. Startle stimuli can accelerate the execution of voluntary motor responses in reaction time (RT) tasks, an effect known as 'StartReact' (Valls-Sole, et al., 2008). If an imperative stimulus, which prompts the initiation of a voluntary motoric action, is accompanied or preceded by a startle stimulus, the response latencies are reduced. Even though the effect can be seen with foreperiods of up to 1500 ms (Carlsen & MacKinnon, 2010), it is most pronounced when the startle stimulus is presented simultaneous with the imperative stimulus. Given that the various modulatory effects exhibit different time courses, both conditions were included in this study to allow inferences about which stage of processing might be affected by the startle stimulus.

4.2 Methods

4.2.1 Participants

Twenty-four male undergraduate students of the University of Trier participated in this study (mean age = 25.45 y, SD = 3.52 y). They were interviewed for present and past medical and/or psychiatric health problems. Participants were excluded for any acute or persistent medical or psychiatric diseases, current medication except the occasional use of pain killers (paracetamol, aspirin, or NSAR), current or past hearing problems (e.g. tinnitus) or impaired vision. To simplify interpretation of responses to erotic slides, only heterosexuals were

recruited. Participants gave their written informed consent and were financially compensated for participation. Experimental procedures were approved by the local ethical committee.

4.2.2 Design

At the beginning of each trial, the participant held his gaze on a black fixation cross in a central position. Target images appeared either in the left or right visual field. Participants were required to maintain fixation until the fixation cross changed colour. With a delay of 200ms from target onset, the fixation cross would change colour for a period of 50ms to indicate gaze direction (and thereby serve as an imperative stimulus): Green demanded a pro-, red an anti-saccade. While a pro-saccade meant to direct the gaze at the image, an anti-saccade meant to look at the image's mirror position, i.e. the opposite blank side of the screen. Both gaze shifts were to be performed as quickly as possible. The target image appeared for a total of 1000ms, the trial ended with the offset of the target image. Three different startle conditions were utilized: 'Startle-SOA -500ms' - 500ms before imperative stimulus onset, 'Startle-SOA 0ms' - synchronous with imperative stimulus onset, and no startle as a control condition (see Figure 5).

A total of 162 trials were presented in pseudo-randomized order. The inter-trial interval (ITI) was randomized between 4-6 sec.
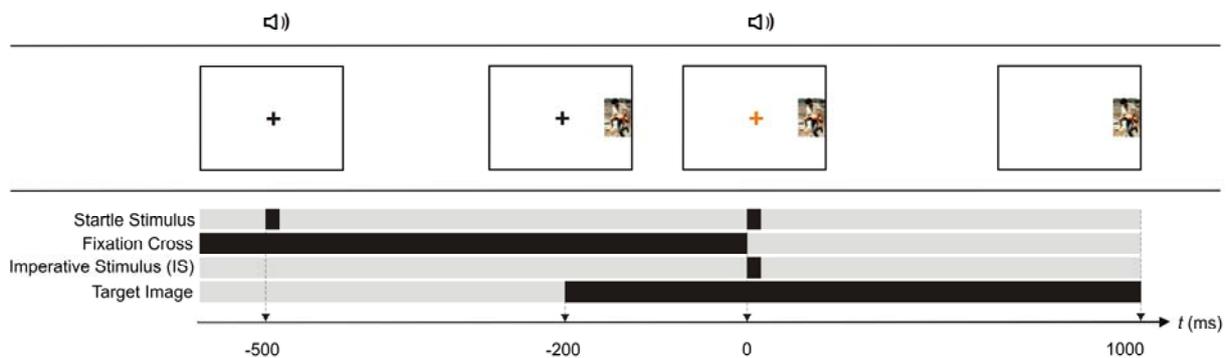

Figure 5:   Experimental protocol.

4.2.3 Procedure

Before the experiment started, the participant gave written, informed consent and was assessed for exclusion criteria. Colour vision was measured with a colour perception test (Broschmann & Velhagen, 1985). Subsequently, the participant was seated in front of a height-adjustable table. The eye tracker was fitted with a head- and chin rest to minimize head movements and keep the distance to the screen constant. The experimenter attached the electrodes for electromyographic recording and calibrated the eye tracker. He informed the participant that the experiment would start immediately and adjusted the headphones. The experimenter left the room and started the experiment from a control room. Throughout the experiment, the participant was able to communicate with the experimenter via an intercom system.

Prior to stimulus presentation, experimental instructions were given in written form on the screen. The participant was told to contact the experimenter in case of uncertainties. The experiment started with a practice block of eight test trials. These trials were identical to experimental trials, with the only exception that grey rectangular forms instead of emotional pictures were used as stimuli. The purpose was to accustom the participant to the task, to check whether the task was correctly understood, and to serve as habituation trials for the startle stimulation (Blumenthal, et al., 2005). These trials were not included in further analysis. Subsequent to the practice trials, the participant was asked if he fully understood the task or had further questions. After total comprehension of the task was ensured, the acquisition phase started.

4.2.4 Apparatus and Materials

4.2.4.1 Stimulus display

The stimuli were presented on a 19 inch flat screen monitor (1280 × 800 resolution, 150 Hz refresh rate). The monitor was positioned at a distance of 60cm from the participant's eyes. Images were presented peripherally, in an upright position (resolution: 230 × 344, visual angle: 6.48° × 9.78°) against a white background. The horizontal distance between the fixation cross (center of the screen) and the center of the picture was 14.61 ° of visual angle, to the left or right.

4.2.4.2 Visual Stimuli

Thirty-six photographic images of unpleasant, neutral, and pleasant scenes were used. Unpleasant images depicted scenes of threat, disgust, and mutilation, neutral pictures displayed scenes and objects such as household items or furniture, pleasant images depicted sport scenarios and erotic nudes. Unpleasant images, neutral images, and sport scenes were selected from the International Affective Picture System (Lang, Bradley, & Cuthbert, 2008), and erotic images were selected from an existing image set used in previous studies (Lass-Hennemann, et al., 2010; Lass-Hennemann et al., 2011). The selection of images was based on previous ratings of valance and arousal. To evaluate the experimental manipulation, each image was rated on these two dimensions by the participants at the end of our study.

Low-level image features (luminance, contrast, saturation) and image complexity were matched between categories with MATLAB 7.12 (MathWorks, Natick, MA). To adjust low level image features, we used the SHINE-Toolbox for MATLAB (Willenbockel et al., 2010). Image complexity was measured by employing a method based on the compression rate of previously generated saliency maps (Da Silva, Courboulay, & Estraillier, 2011). We generated 'Itti, Koch, Niebur saliency maps' for each image (Itti, Koch, & Niebur, 1998) by using the GBVS-algorithm (Harel, Koch, & Perona, 2007). The size values of the compressed saliency maps were submitted to an analysis of variance (ANOVA), that yielded no significant differences between categories ($F_{2,33} = .67$; $p = .55$).

4.2.4.3 Eye tracking

Eye movement data were recorded with an SMI iView-X HiSpeed 500 (500 Hz sampling rate, spatial accuracy better than 0.5°). The eye tracker was mounted on a height adjustable table, at a distance of 60cm to the monitor.

4.2.4.4 Startle Stimulation

Startle stimuli were acoustic white noise probes (105 dB, 50 ms duration, instantaneous rise time, binaural stimulation) presented via audiometric headphones (Holmco PD-81, Holmberg GmbH & Co. KG, Germany).

4.2.5 Data acquisition and Analysis

4.2.5.1 Eye tracking

Horizontal and vertical gaze data were recorded as analogue data, saved on hard disc and analyzed off-line. Analysis was made with a C++ based, semi-automated program.

Saccadic onset and peak (reaching target location) were automatically detected within a range of 200-1000ms past imperative stimulus-onset. All trials were manually checked and artefact corrected when necessary. Trials were excluded from analysis when a blink was detected 200ms prior to imperative stimulus-onset or when eyes were not focussed on the central fixation cross at target-onset. When a saccade was initiated in a time window between -200ms before and 80ms after imperative stimulus-onset, or when the saccade was directed to the wrong side, the trial was considered to be an error trial.

We calculated the reaction time as the latency between the onset of the imperative stimulus and the onset of the saccade and the saccade duration as the time between saccade onset and first fixation.

4.2.5.1 Startle analysis

Electrodes for EMG recording of the m. orbicularis oculi were attached below the participant's right eye at an inter-electrode distance of 1.5 cm. The EMG-signal was recorded on hard disk with a BIOPAC MP 150 system and an EMG 100C amplifier via Tyco Healthcare H124SG electrodes at 16 bit resolution and 1 kHz sampling rate. Hardware band-pass filter settings were 10 to 500 Hz, followed by a 28 Hz software high-pass filter (van Boxtel, et al., 1998). The raw signal was rectified and integrated online with a time constant of 10 ms (Blumenthal, 1994). The data of seven participants had to be discarded from further analysis because of data acquisition problems.

The EMG-startle responses were analyzed offline with a C++ based, semi-automated program. Startle response magnitude was defined as difference between peak and baseline signal. The integrated algorithm identified peak in a time interval between 20-150 ms after stimulus onset. Baseline was assessed 50 ms prior to stimulus onset (Lass-Hennemann, et al., 2010). Each response was manually confirmed and corrected for non-responses and artefacts. Non-responses (cases with no discernable response) were set to zero and included in the analysis (25.4 % of all trials). Cases with electrical and physiological artefacts (such as voluntary or spontaneous eye blinks coinciding with the startle stimulus, or trials with excessive background noise or multiple peaks) were excluded from analysis (0.7 % of all trials). Response magnitude was averaged across trials for each condition (Blumenthal, et al., 2005).

4.2.5.2 Subjective Ratings

After the data acquisition phase, the participant was asked to rate the previously presented images on the dimensions of valence and arousal. Each image was displayed together with a five-digit scale for both dimensions. The rating was based on the 'Self-Assessment Manikin' rating system (Lang, et al., 2008).

4.2.6 Statistical Analysis

The saccade reaction data were analyzed in a 2 (saccade direction: Pro-saccade vs. Anti-saccade) × 3 (startle stimulus: -500ms SOA vs. 0ms SOA vs. no) × 3 (valence: positive vs. neutral vs. negative) within-subjects ANOVA. The EMG response data were analyzed for the startle stimulus condition (--500 SOA vs. 0ms SOA) in a t-test. For the Startle-SOA 0ms condition, the factor 'valence' was analyzed in a one-factorial ANOVA (positive vs. neutral vs. negative). Significant interactions were analyzed with Bonferroni-adjusted post-hoc t-tests. Response magnitude between both startle stimulus conditions was compared with a paired-sample t-test. Subjective rating data for 'valence' and 'arousal' were subjected to a one-factorial ANOVA (positive vs. neutral vs. negative). Reported *p*-values for factors with more than two conditions are 'Greenhouse–Geisser' corrected. The critical alpha-level was set to .05 in all analyses.

4.3 Results

4.3.1 Subjective Ratings

A significant difference between image categories was found for the valence dimension (F $_{2,42}$ = 147.78; p<.001; η²=.88) and the arousal dimension (F $_{2,42}$ = 85.7; p<.001; η²=.80). Positive images were rated as more pleasant than neutral images, and neutral as more pleasant than negative images (M= 1.78 [neg], 3.27 [ntr], 4.06 [pos]). Negative and positive images were both rated as more arousing than neutral images, and scores for negative and positive images were equivalent (M= 3.64 [neg], 1.46 [ntr], 3.63 [pos]).

4.3.2 Saccadic RTs

A significant main effect of startle stimulus condition was found (F $_{2,42}$ = 24.55; p<.001; η²=.54). Saccades were initiated faster in the preliminary startle-SOA -500ms condition, compared to startle-SOA 0ms and no-startle conditions. The main effect of saccade

direction was significant (F $_{2,42}$ = 4.84; p<.05; η²=.19), in that pro-saccades were initiated faster than anti-saccades. The main effect of valence was significant (F $_{2,42}$ = 4.08; p<.05; η²=.16), with reactions to neutral images being faster compared to positive or negative images. However, the significant interaction of the factors Saccade Direction × Valence (F $_{2,42}$ = 6.98; p<.05; η²=.25), showed that the slowest reactions in the pro-saccade condition were to negative images (t $_{22}$ = 3.88; p<.05), but in the anti-saccade condition the slowest reactions were to positive images (t $_{21}$ = 2.46; p<.05; see Figure 6).
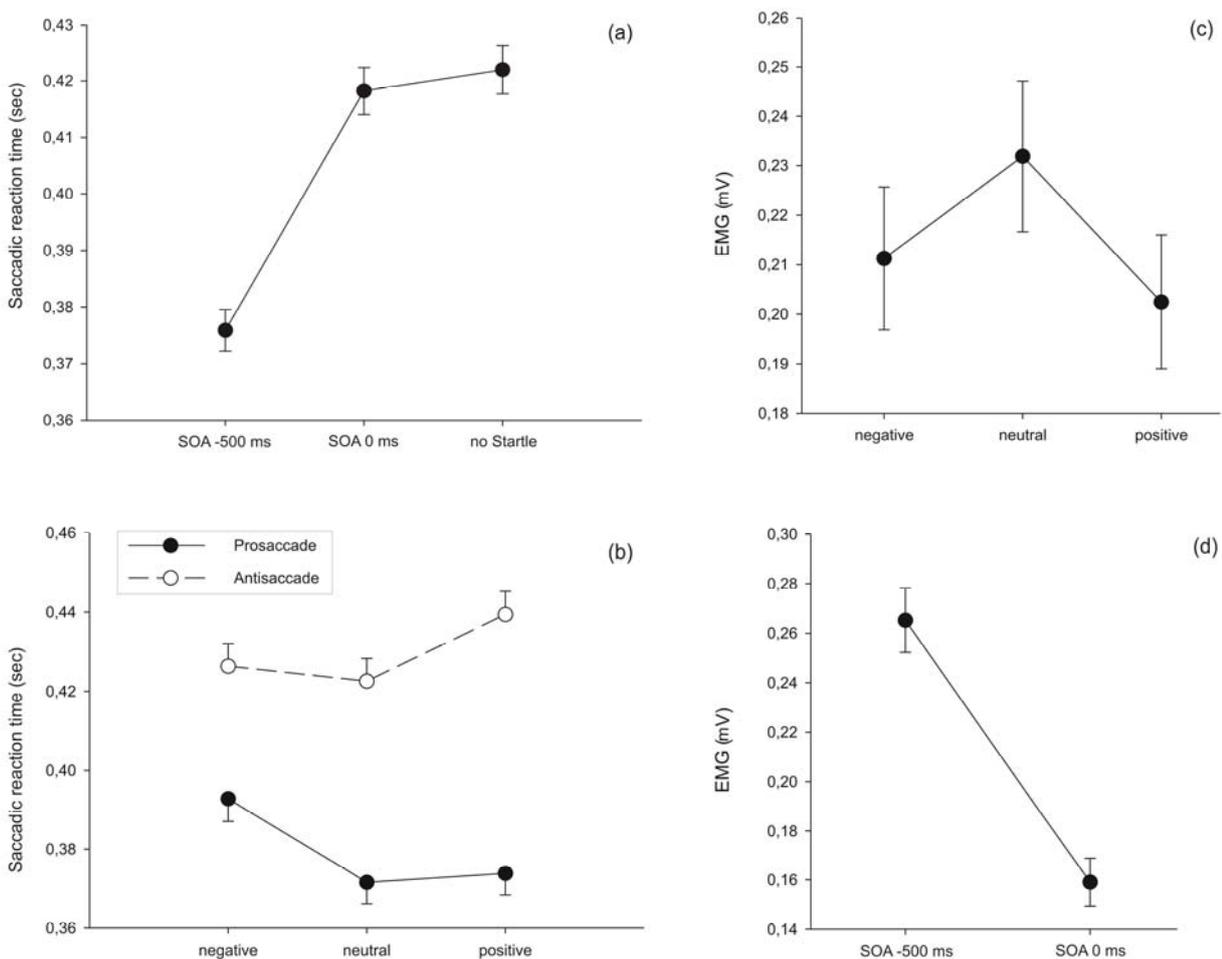

Figure 6:   Saccadic reaction times (mean, SD) for (a) the startle SOA conditions and (b) for the interaction between target valence and saccade direction and startle EMG Magnitude (mean, SD) for (c) target valence in the startle B condition and (d) between startle SOA conditions

4.3.3 Saccade duration

The only significant difference was found for the factor 'saccade direction' ($F_{2,44} = 64.08$; $p<.001$; $\eta^2=.75$): Pro-saccades had a significantly shorter duration between saccade onset and first fixation than anti-saccades.

4.3.4 Saccade error rate

The only significant difference was found for the factor 'startle stimulus' ($F_{2,44} = 13.88$; $p<.001$; $\eta^2=.39$), with significantly lower error rates in the 'Startle-SOA -500ms' -condition (M= 1.78 [Startle-SOA -500ms], 3.27 [Startle-SOA 0ms], 4.06 [no startle]).

4.3.5 Startle EMG Response

Average response magnitude in the 'Startle-SOA 0ms' condition was significantly lower compared to that in the 'Startle-SOA -500ms' condition ($t_{16} = 4.12$; $p<.05$). The effect of the factor 'valence' was investigated in the Startle-SOA 0ms condition only (since the startle stimulus preceded picture onset in the Startle-SOA -500ms condition, no effect of valence would be possible in that condition). The effect of 'valence' was significant when comparing the response magnitude in the 'Startle-SOA 0ms'-condition ($F_{2,38} = 3.58$; $p<.05$; $\eta^2=.17$). Response magnitude was significantly higher when neutral pictures were shown, compared to positive and negative pictures.

4.4 Discussion

The present experiment investigated the effects of acoustic startle stimulation on the processing of visual emotional stimuli. We included two startle conditions with different SOAs (500ms before and simultaneous with an imperative stimulus) in a saccadic reaction task.

We found an effect of the acoustic startle probe on saccadic reactions. Saccades were initiated faster when preceded by a startle stimulus. This effect is consistent with the literature on accessory stimuli: The startle stimulus could have functioned as an accessory stimulus, which would have resulted in increased alertness for the upcoming task (Hackley et al., 2009). Unlike Castellote et al. (2007) the effect was not found when the startle probe was delivered

simultaneous with imperative stimulus-onset. While participants in that study performed reflexive saccades to peripherally appearing targets, our task required voluntary, effortful saccades. The externally guided saccades in that study resembled a simple RT task. In contrast, our manipulation was a more complex choice RT task, which needed further processing and is therefore less prone to the StartReact effect (Oude Nijhuis, et al., 2007; Reynolds & Day, 2007). Castellote et al. interpreted the response acceleration as an effect of the startle stimulus on motor preparation. However, with a foreperiod of 500ms, sensory and attentive processes or a surge in arousal are likely mechanisms for RT-shortening as well. We found no significant differences for saccade duration, indicating that the motoric execution of saccades was not affected by startle presentation. Furthermore, there was no trade-off between speed and accuracy. Instead, responses in the early startle condition were not only faster, but also had reduced error rates. Therefore, mere response guessing or a preprogramming of the motor response seems unlikely. Hackley and Valle-Inclán (2003) proposed response selection as a candidate mechanism for accessory stimulus effects, which would fit well with the improved performance in our rather complex choice-reaction task. The absence of an accelerating effect in the 'Startle-SOA 0ms' condition could be attributed to the lack of a foreperiod (Diederich & Colonius, 2008). If the simultaneous startle coincided with response selection, distracting interference could have outweighed a possible accelerating effect in this condition.

Compared to the early 'Startle-SOA -500ms' condition, the average startle EMG magnitude was reduced in the simultaneous 'Startle-SOA 0ms' condition. This attenuation can be explained by the concept of 'prepulse inhibition' (Graham, Putnam, & Leavitt, 1975): The target pictures, which appeared 200ms prior to startle onset, served as a prepulse. Compared to neutral images, EMG magnitudes were even more decreased for emotional images in the 'Startle-SOA 0ms' condition. This effect seems to rely on the arousal induced by the visual stimuli, since it was independent of valence. The averaged EMG magnitudes in the 'Startle-SOA 0ms' condition were decreased for emotional pictures. Since these pictures potentially served as prepulses, the prepulse inhibition effect was stronger for emotional pictures (Bradley, et al., 1993). Since these valenced pictures were also more arousing, this can be taken as evidence that the arousal information of the parafoveal target images was processed.

As expected from previous literature, anti-saccades were initiated with longer response latencies than pro-saccades (Gilchrist & Proske, 2006; Kissler & Keil, 2008; Morand, Grosbras, Caldara, & Harvey, 2010). Whereas both forms of attentional gaze control converge in the case of a pro-saccade, an anti-saccade induces a conflict situation. While the target

draws attention in a bottom-up way, the automatic saccade needs to be actively inhibited. By drawing on executive resources, the anti-saccade is described as more challenging and, as we found in this study, takes more time to be performed (Everling & Fischer, 1998).

Furthermore, we found an interaction between saccade type and stimulus valence. Previous studies have demonstrated that emotional content, even when presented to the parafovea, can influence saccadic reactions. However, the gaze pattern in our study reflects quite specific effects of valence: Reactions were slowed for saccades towards negative and away from positive targets. Many studies that required saccadic responses to emotional targets have found responses towards negative stimuli to be faster than towards neutral or positive stimuli, which is explained by an attentional bias to potentially dangerous situations (Bannerman, Milders, de Gelder, & Sahraie, 2009; Gerdes, Pauli, & Alpers, 2009; Wieser, Pauli, & Muhlberger, 2009). However, an attentional bias to negative stimuli is not without exception. Other studies have found RT shortening for emotional content, irrespective of valence, or faster reactions to positive stimuli (Bannerman, et al., 2009; Kissler & Keil, 2008; Nummenmaa, et al., 2009). We assume that the slowed responses in our study reflect the tendency to avoid those pictures. This would be in line with results from a study using the free-viewing paradigm: When angry, fearful, or happy faces were presented simultaneously with neutral faces, participants actively avoided looking at the negative images (Becker & Detweiler-Bedell, 2009). While the standard pro-/ anti-saccade paradigm requires immediate responses to target stimuli, the target stimuli in our study were already present for 200ms before a response was required. We might speculate that an initial capture of attention for negative stimuli is followed by the tendency to avoid them. Indeed, the "vigilance-avoidance"- hypothesis would predict exactly such a pattern (Mogg, Bradley, Miles, & Dixon, 2004).

Divergent effects have been reported for anti-saccades away from emotional stimuli. While some studies found slower responses away from negative stimuli (Wieser, et al., 2009), others reported slower responses away from positive stimuli (Phillips, 2009). In our study, anti-saccades took longer when positive targets were presented. Again, this would be indicative of approach behaviour in the sense of the approach-avoidance-concept: The tendency of directing the gaze to the positive content needs to be overridden while an effortful, opponent response is initiated.

Contrary to our initial hypothesis, startle did not interact with valence in our saccadic task. One possible explanation is that the foreperiod of 500ms was too short to induce emotion-specific effects. Even though affective startle modulation was found under certain

conditions with lead times as low as 250ms (Vanman, Boehmelt, Dawson, & Schell, 1996) or 300ms (Gard, et al., 2007; Stanley & Knight, 2004), these early effects are not commonly found. In the standard paradigm, the emotional foreground stimulus is presented with lead times of more than one second. One suggestion is that these early emotional effects draw on processes other than motivational priming (Filion, et al., 1998). We speculate that, even for the 'Startle-SOA -500ms' condition, motivational priming effects did not have enough time to build up. It would therefore be of interest to investigate the effect of even longer foreperiods. On the other hand, unlike an emotional picture, a startle stimulus extends over a brief moment in time. By the time of the behavioural response, motivational priming effects might have vanished. Touching on the 'cardiac defence' paradigm, one could think of extending the duration of the white noise exposure to overcome this problem (Ramirez, Sanchez, Fernandez, Lipp, & Vila, 2005; Sanchez et al., 2009).

Some further limitations of the study have to be addressed. Since we included only males in this study, female responses in such a setting need to be investigated as well. While women might respond more to startle (Kofler, Müller, Reggiani, & Valls-Solé, 2001), the gaze pattern in response to emotional pictures might be different as well (Bradley, Codispoti, Sabatinelli, & Lang, 2001). With this study we cannot disentangle the accessory stimulus effect from those that are unique to startle stimuli. Therefore, it would be of interest to see how non-startling accessory stimuli affect saccade programming in such a paradigm.

In conclusion, startle was shown to speed saccadic reactions and improve accuracy when presented with sufficient lead time before an imperative stimulus. Furthermore, we could demonstrate that emotional information of parafoveally presented images has a moderating influence on prepulse inhibition, perhaps due to increased arousal of those images. We hypothesized that startle, because of its defensive character, would differentially affect responses depending on target valence. However, the results do not provide support for a valence dependent effect of startle on oculomotor reactions. While startle responses are affected by emotional foreground stimuli, responses to emotional stimuli seem to be unaffected by previous startle stimuli. Independent of the startle related effects, we found a remarkable interaction pattern between saccade direction and target valence which suggests that approach-avoidance motivation can be extended to gaze behaviour.

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Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbst verfasst und keine anderen als die angegebenen Hilfsmittel verwendet habe. Zudem wurde die Arbeit an keiner anderen Universität zum Erlangen eines wissenschaftlichen Grades eingereicht.

Trier, den 20.06.2013

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Christian Eric Deuter