**Emotion-Modulated Startle Reflex during Reappraisal: Probe Timing and Behavioral Correlates**

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Abstract

Down-regulation of negative emotions has been shown to reliably inhibit the emotion-modulated startle reflex, but it remains unclear whether **the timing of the startle probe influences the quantification of emotion regulation with this measure**. Moreover it is not known, whether the degree of startle inhibition corresponds to the subjective attenuation of negative emotions. Therefore the two main goals of the study were first to systematically analyze the effect of probe time on startle inhibition. Second, we aimed to explore the association between subjectively perceived down-regulation of arousal and valence and the degree of startle inhibition. We presented negative and neutral pictures to *N* = 47 participants. Pictures were paired with the instruction to reappraise or to maintain the emotions elicited by these pictures. Probes were delivered at three different times during a 12.5 s regulation phase and the startle response was measured with electromyography. Valence and arousal ratings were assessed after each trial. Results revealed no significant impact of probe time on startle inhibition during reappraisal. Startle inhibition and perceived down-regulation of arousal were significantly and positively correlated, whereas perceived down-regulation of valence was not. The results provide important implications for future studies in terms of startle probe timing and shed light onto the interpretation of startle inhibition as an indicator of subjective attenuation of negative emotions.

*Keywords:* emotion regulation, reappraisal, startle reflex, arousal, psychophysiology

Emotions add flavor to life and substantially shape the adaptive and survival responses to emotional stimuli (Ekman & Davidson, 1994). Emotions are organized in an appetitive and a defensive motivational system (Lang, Simons, & Balaban, 1997) based on valence and arousal dimensions. Viewing of negative pictures activates the aversive motivational system and defensive reflexes, which vary with the intensity (i.e. arousal) of the stimulus (Bradley, Codispoti, Cuthbert, & Lang, 2001). The startle response is a defensive reflex and is typically measured as the contraction of the *orbicularis oculi* muscle in response to auditory probes in human studies (Lang, Bradley, & Cuthbert, 1990). A substantial body of work (Davis, Campeau, Kim, & Falls, 1995; Lang et al., 1990) has demonstrated that startle amplitudes are increased and decreased in negative and positive emotional states, respectively (i.e. *emotion-modulated* startle), with responses being more potentiated and inhibited during viewing of highly arousing stimuli (Bradley, Codispoti, Cuthbert, et al., 2001; Vrana, Spence, & Lang, 1988a).

Humans can purposefully control the type, intensity and occurrence of their emotions in a context-dependent manner, which is known as emotion regulation (Gross, 1999, 2002). The most prominent and well-studied emotion regulation strategy is reappraisal, which is implemented before the behavioral response has fully unfolded (Gross, 1998). The emotion-modulated startle can be used as a specific measure of reappraisal because it is inhibited when down-regulating and potentiated when up-regulating negative emotions (Adolph & Pause, 2012; Bernat, Cadwallader, Seo, Vizueta, & Patrick, 2011; Conzelmann, McGregor, & Pauli, 2015; Dillon & LaBar, 2005; Driscoll, Tranel, & Anderson, 2009; Grillon, Quispe-Escudero, Mathur, & Ernst, 2015; Jackson, Malmstadt, Larson, & Davidson, 2000; Lee, Shackman, Jackson, & Davidson, 2009; Lissek et al., 2007; Piper & Curtin, 2006). It has been demonstrated that the down-regulation of negative emotions through reappraisal involves a significant reduction of amygdala activity (Buhle et al., 2014). Since projections from the amygdala modulate the startle reflex (Davis, 2000), down-regulation of the amygdala likely mediates startle inhibition with reappraisal.

Several questions however remain unanswered. As reappraisal processes are dynamic, the startle response may change as a function of probe timing. Previous studies demonstrated greater startle potentiation for probes delivered later compared to those delivered earlier during a 6-12s period of emotional picture viewing (Bradley, Codispoti, Cuthbert, et al., 2001; Sutton, Davidson, Donzella, Irwin, & Dottl, 1997). When it comes to reappraisal, it is unclear whether probe timing has an impact **on its meaningful quantification with the emotion-modulated startle reflex**. According to the implementation and maintenance model (Kalisch, 2009; Paret et al., 2011) reappraisal is divided into an early and late phase. In the early phase, participants choose and implement a strategy, whereas in the late phase they maintain the strategy in working memory and monitor its success. In light of this, reappraisal might need several seconds until it effectively reduces negative emotions. Thus, startle modulation may become more pronounced as soon as the maintenance of reappraisal predominates. In line with this, small decreases of the startle amplitudes were observed when probes were delivered 3 seconds into the regulation phase, but large decreases were observed when probes were delivered 8-11 seconds into the regulation phase (Dillon & LaBar, 2005; Jackson et al., 2000). **However, these studies did not directly test whether effects of early versus late probes were significantly different during emotion down-regulation compared to the control condition.** **Moreover, they did not specifically assess reappraisal but rather participants were free to choose a cognitive strategy they found most useful.** Another study delivered the startle probe 2 seconds into the reappraisal phase and reported non-significant startle inhibition (Eippert et al., 2007). In addition, most pronounced amygdala down-regulation was observed after probe presentation, suggesting that the probe might have been given too early to reliably detect reappraisal effects**. In contrast, a similar study found smaller startle magnitudes when participants down-regulated emotions to pictures as compared to the up-regulating condition within the early emotion regulation period but not within the late emotion regulation period (Adolph & Pause, 2012). In that study, the regulation period was only five seconds long and there was no control condition, so the effects are not directly comparable to those reported by Dillon & LaBar (2005), Eippert et al. (2007) and Jackson et al. (2000). Our study was specifically designed to test the impact of startle probe timing on the quantification of emotion down-regulation through the emotion-modulated startle reflex. Particularly, we aimed at testing three different startle probe times evenly distributed over a 12 seconds time course of reappraisal, which is critical, as such an approach may help identifying startle probe timing as one potential factor contributing to existing contradictory findings and to avoid** misinterpretation of non-significant effects in future reappraisal studies.

A second issue concerns the question whether the emotion-modulated startle reflex as an objective measure of emotion down-regulation is correlated with subjective attenuation of negative emotions. One way to measure subjective attenuation of negative emotions is to assess the perceived valence of one’s emotions and one’s level of arousal after each regulation trial. The difference of arousal as well as valence between regulation and control condition then serves as a measure of subjective attenuation of negative emotions. Startle modulation by emotional stimuli has been shown to be sensitive to changes in both perceived stimulus valence and emotional arousal during picture viewing (Lang, Bradley, & Cuthbert, 1997; Vrana et al., 1988a; Witvliet & Vrana, 1995). However, studies have found that the instruction to decrease emotions attenuated startle responses forboth negatively and positively rated pictures (Bernat et al., 2011; Conzelmann et al., 2015; Dillon & LaBar, 2005), suggesting that startle reflex in response to emotion regulation may vary with perceived arousal, but not with valence. Few studies have assessed valence and arousal ratings in addition to the emotion-modulated startle (Bernat et al., 2011; Conzelmann et al., 2015; Dillon & LaBar, 2005) and only one of these studies (Bernat et al., 2011) assessed them during the experimental task, but the latter did not provide an analysis of covariance. We are not aware of any study that directly explored the relationship between subjective attenuation of valence and arousal of negative emotions and the degree of startle inhibition during reappraisal.

To address these questions, we conducted a reappraisal experiment with early, middle and late probe presentation (2s, 7s and 12s into the regulation phase). Furthermore, we assessed how negative and how aroused participants felt after each trial. At the end of the experiment, we assessed the habitual use of cognitive emotion regulation strategies. **On the basis of both the implementation and maintenance model and previous findings assessing both early and late startle probes (Dillon & LaBar, 2005; Jackson et al., 2000), we hypothesized that** **the difference of startle amplitudes in the reappraisal versus the control condition significantly increases with startle probe time** (i.e. 2s<7s<12s). We also hypothesized that down-regulation of arousal significantly correlates with startle inhibition through reappraisal. Another goal of our study was to explore whether the frequency to which participants engage in reappraisal and other cognitive emotion regulation strategies in daily life would be associated with startle inhibition, as previous studies have shown that both difficulties with emotion regulation and the frequent use of reappraisal are associated with alterations in psychophysiological responding (Mauss, Cook, Cheng, & Gross, 2007; Williams et al., 2015).

**Methods**

**Participants**

47 healthy right-handed females (age: *M* = 23.9, *SD* = 5.5) participated in this study. Only females were studied to avoid confounding effects of gender differences (Bradley, Codispoti, Sabatinelli, & Lang, 2001). Exclusion criteria checked beforehand in a telephone interview comprised any mental disorder according to the DSM-V criteria, any history of psychiatric or neurological treatment, drug use, regular intake of medicine, current pregnancy and a BMI below 16.5. All participants had normal vision and hearing and were fluent in German. The study was approved by the Ethics Committee of the Medical Faculty Mannheim/ Heidelberg University and was conducted according to the Declaration of Helsinki. After full explanation of the study, all subjects gave written informed consent prior to participation and were paid 30 Euro for participation.

**Apparatus and Procedure**

To increase motivation, subjects were told at the beginning of the experiment that an additional amount of 15 Euro would be paid to the top 25% regulators, as determined by their “physiological responses” to the stimuli. At the end of the experiment, all participants received the additional 15 Euro and were debriefed (cf. Jackson et al., 2000). Participants were instructed either to view negative and neutral pictures without modifying their emotions or to down-regulate their feelings toward negative pictures by means of reappraisal strategies. Reappraisal strategies comprised distancing oneself from the depicted content or to reinterpret the depicted content in a positive or neutral manner. Oral practice trials were conducted before the computer task until the participant was able to apply reappraisal properly. Furthermore, participants were instructed not to turn away their gaze or to close their eyes, nor to focus exclusively on non-emotional parts of the picture. For a detailed description of the experimental procedure see Fig. 1. The participants’ eyes were tracked by a camera system (SMI BeGaze, Teltow, Germany) to encourage subjects to stay with the task but data were not analyzed. Trial order was pseudorandomized and counterbalanced with no more than two consecutive conditions of the same type. In total, the paradigm consisted of 63 trials (7 trials per condition) and lasted 40 min. After trial 21 and trial 42 the task paused for a couple of minutes to prevent fatigue.

**Picture Stimuli**

Stimuli were taken from the standardized picture series (Lang, Bradley, & Cuthbert, 2008; Marchewka, Żurawski, Jednoróg, & Grabowska, 2014) and were presented with the Presentation software (Neurobehavioral Systems, Berkeley, CA) in semi-randomized order with restriction of no more than two consecutive trials from the same condition, and no more than three consecutive trials with negative pictures. Sets of 42 negative pictures (valence: *M* = 2.36, *SD* = .68; arousal: *M* = 6.86, *SD* = .23) and 21 neutral pictures (valence: *M* = 5.21, SD = .59; arousal: *M* = 2.57, SD = .26) were created (normative ratings based on representative samples (Lang et al., 2008; Marchewka et al., 2014)). **Details about the stimuli we used in our experiment can be derived from the supplement.** Arousal and valence ratings differed significantly between the sets (both *p*s <.001). The pictures were further divided into two sets (balanced for content, valence, and arousal), which resulted in three conditions depending on instruction and picture type: View neutral pictures (LookNeu), view negative pictures (LookNeg) and down-regulate emotions while viewing negative pictures with reappraisal (RegNeg). Assignment of negative picture sets to LookNeg and RegNeg condition was alternated between subjects.

**Measures**

**Emotion-modulated Startle**

The eye blink was measured by electromyogram (EMG). Two Ag-AgCl electrodes were placed on the orbicularis oculi muscle below the left eye, and a ground electrocardiogram electrode was attached on the lower rip bow1. The raw EMG signal was sampled at 1000 Hz, and the gain was amplified by 2000. High-pass (50 Hz) and low-pass (500 Hz) filters were applied to the data with AcqKnowledge software (BIOPAC Systems; Goleta, CA). EMG data were integrated over 10 samples and analyzed offline with Clip, a C++based, semi-automated program (Kinzig, Schulz, Curio, & Schächinger, 2008). Startle response was defined as the difference between peak (20–120 ms after stimulus onset) and baseline (50 ms prior to stimulus onset) signal. Trials including movement artifacts, excessive baseline activity (exceeding 2 standard deviations [SD] above baseline mean), or non-responses (peak < four SD below baseline mean) were excluded (mean % = 9.56 [SD = 6.52]) of all trials across participants). Startle data from six participants were excluded because of excessive noise (more than 30% missing). Finally, amplitudes were z-standardized within participants and transformed to *T*-scores with mean = 50 and SD = 10. Responses were averaged across participants for each condition.

**Cognitive Emotion Regulation Questionnaire (CERQ)**

To measure how frequently participants apply cognitive emotion regulation strategies in daily life, we administered the German adaptation of the Cognitive Emotion Regulation Questionnaire (CERQ;(Garnefski, Kraaij, & Spinhoven, 2002) after the emotion regulation task. The CERQ is a 36-item questionnaire, comprising the following nine conceptually different subscales: *Self-blame, Acceptance, Planning, Positive Refocusing, Rumination, Positive Reappraisal, Putting into Perspective, Catastrophizing and Other-blame.*

**Postexperimental questionaires**

After the emotion regulation task, participants indicated the subjectively experienced regulation success on a 9-point Likert scale ranging from 1 = not at all to 9 = very much. Questions were adapted from Gallo, Keil, McCulloch, Rockstroh, and Gollwitzer (2009): 1. *How much have you tried to reduce your negative feelings?*; 2. *How difficult was it to reduce your negative feelings?*; 3. *How well did you succeed in realizing the goal expressed in the reappraisal instruction?* Moreover, participants were asked to indicate to what percentage they used the following strategies during the regulation conditions: 1. Reappraisal, 2. Distancing, 3. Distraction, 4. Relaxation, 5. Mindfulness, 6. Attentional deployment, 7. Other strategies. Details and results about the used emotion regulation strategies can be derived from the supplement (see SXXX).

**Data Analysis**

Data analysis was performed using SPSS version 24 (SPSS Inc., Chicago, IL, USA). Before interaction analysis of emotion regulation and startle probe timing, we subtracted the mean amplitudes of the LookNeu conditions from the RegNeg and from the LookNeg conditions. We then conducted a repeated-measures ANOVA with 2 *condition* (RegNeg, LookNeg) x 3 *probe timing* (2s, 7s, 12s) levels. The Greenhouse-Geisser correction was used to correct sphericity, and corrected *p* values are reported. Additionally, to analyze the correlation between startle inhibition and subjective attenuation of negative emotions, we calculated difference scores (LookNeg minus RegNeg) for startle amplitudes (*T*-scores) and valence and arousal ratings. Furthermore, CERQ subscales were correlated with startle inhibition using Pearson’s correlation coefficient and assessed significance at the *p* < .05 level.

**Results**

**Ratings (Manipulation Check)**

All participants adhered to the reappraisal task as evidenced by the post-hoc success ratings (see Table S1 for means and SDs for each question).

Induction of negative emotions was successful, evidenced by paired t-tests showing that arousal and valence ratings were significantly higher in the LookNeg (arousal: *M* = 4.31, *SD* = .23; valence: M = 5.96, SD = .22) than in the LookNeu (arousal: *M* = 1.57, *SD* = .10; valence: *M* = 2.89, *SD* = .18) condition [arousal: *t*(46) = 12.25 , *p* < .001; valence: *t*(46) = 14.51, *p* < .001]. Moreover, all participants were able to down-regulate their level of arousal and negative emotional state with reappraisal as evidenced by lower arousal ( *M* = 3.18, *SD* = .18) and less negative/more positive valence (*M* = 4.85, *SD* = .20) in the RegNeg condition compared to the LookNeg condition [arousal: *t*(46) = -6.23, *p* < .001; valence: *t*(46) = -7.71, *p* < .001].

**Emotion-modulated Startle**

The 2 (condition) x 3 (startle probe) repeated measure ANOVA revealed a significant main effect of *condition*, ***F*(1,40) = 6.11, *p* = .02, eta2 = .13.** The main effect of *probe timing* was not significant, ***F*(2,80) = .67, *p* = .60, eta2 = .01.** Contrary to our hypothesis, the interaction *condition* x *probe timing* was also not significant, ***F*(2,80) = .51, *p* = .60, eta2 = .01 (Fig.2a).**

**Correlations**

***Arousal and Valence Ratings***

Pearson’s correlational analysis showed that the difference scores (LookNeg minus RegNeg) of startle amplitudes were significantly and positively associated with difference scores (LookNeg minus RegNeg) of arousal ratings **(*r* =.41, *p* = .01, *n* = 41)**, indicating that participants who showed a stronger subjective attenuation of arousal also showed a stronger inhibition of the startle amplitude during reappraisal (Fig. 2b). In contrast, difference scores (LookNeg minus RegNeg) of startle amplitudes did not significantly correlate with difference scores of valence ratings **(*r* = .26, *p* = .11, *n* = 41; Fig. 2c**), suggesting that the inhibition of startle during reappraisal is not predictive for the subjective attenuation of valence.

***CERQ***

For descriptive statistics on CERQ subscales see Table S2 (supplement). Internal consistency of the CERQ was good, as evidenced by Cronbach’s alpha = 0.77. Correlations between difference scores (LookNeg minus RegNeg) of startle amplitudes and CERQ subscales and success ratings (Table S3, supplement) were not significant (all *p*s > .05).

**Discussion**

The primary goal of this study was **to examine whether effects of reappraisal assessed with the startle eye-blink increase with startle probe time.** In addition, we analyzed whether the degree of startle inhibition during reappraisal was predictive for the subjective attenuation of negative emotions. Results demonstrated that subjects successfully reduced negative emotions evidenced by startle inhibition, arousal, valence and post-hoc success ratings. Contrary to our expectations, startle inhibition was independent of probe timing. In other words, whether probes were delivered at 2, 7 or 12 seconds into the reappraisal phase did not significantly affect the assessment of emotion regulation. In accordance with our second hypothesis, startle inhibition was significantly and positively correlated with subjective attenuation of arousal but not with subjective reduction of valence.

With our study we were able to replicate the finding that down-regulating negative emotions with cognitive emotion regulation strategies inhibits emotion-modulated startle (Conzelmann et al., 2015; Grillon et al., 2015; Jackson et al., 2000; Lee et al., 2009; Lissek et al., 2007; Piper & Curtin, 2006). This corresponds to a recent meta-analysis from our group, which revealed that the emotion-modulated startle is a reliable measure of emotion regulation (Zaehringer, Jennen-Steinmetz, Schmahl, Ende, & Paret, submitted). Although temporal differences were not significant, a visual inspection of results shows that startle probes delivered at ≥7s are useful to quantify reappraisal effects (see Fig. 2). Descriptively, startle probes delivered at 2 s produced smaller effects and might be less sensitive than later probes (Dillon & LaBar, 2005; Eippert et al., 2007; Jackson et al., 2000), though these differences are not significant.

Startle inhibition during reappraisal correlated with the perceived down-regulation of arousal. This finding is in line with our second hypothesis, which was based on previous literature suggesting that the regulation effect on the emotion-modulated startle follows the pattern of variations in arousal of pictures (Bernat et al., 2011; Dillon & LaBar, 2005). As pointed out earlier, **emotions are organized around a valence and arousal dimension (Lang et al., 1997) with the valence dimension determining approach or avoidance motivation, and the arousal dimension determining the degree of activation of approach and avoidance. Thus, one interpretation of our finding might be that reappraisal of negative emotions does not elicit a transition from avoidance to approach but rather reduces the intensity of avoidance behavior. The present result moreover demonstrates that the reduced intensity of avoidance measured with emotion-modulated startle directly reflect changes in perceived levels of arousal.** Efferent pathways from the amygdala, found to modulate startle (Davis, 1992), are involved in regulating arousal-related responses to aversive stimuli (LeDoux, Iwata, Cicchetti, & Reis, 1988; Reyes, Carvalho, Vakharia, & Van Bockstaele, 2011). Hence, subjective arousal as well as avoidance motivation may be down-regulated due to amygdala inhibition, and the shared neural mechanism may account for the finding. Alternatively, it could be that subjects may have rated arousal based on self-observation of startle intensity.

In contrast, we found no significant correlation between the perceived down-regulation of valence and startle inhibition. The result raises the possibility that in the context of emotion regulation the affective modulation of the startle response might be a particularly strong indicator of one’s level of arousal rather than a measure of the valence of one’s emotional state. This assumption is complemented by prior research on emotional picture viewing showing that both the degree of affective modulation of the startle response and amygdala activity become more pronounced as the level of subjective arousal of pictures increases (Cuthbert, Bradley, & Lang, 1996; Phan et al., 2004; Sabatinelli, Bradley, Fitzsimmons, & Lang, 2005).

No correlations were found between startle inhibition and CERQ subscales, suggesting that startle inhibition during reappraisal might not be indicative for a frequent use of reappraisal and other forms of cognitive emotion regulation strategies. This is contrary to previous studies showing that people who frequently engage in reappraisal show different psychophysiological responding in aversive situations than people who use reappraisal less frequently (Mauss et al., 2007; Memedovic, Grisham, Denson, & Moulds, 2010).

The study is not without limitations. To limit attentional demands and fatigue due to long task duration, **ITI was short and** startle probes were presented in every trial. As a result, late probe types B and C, in particular, were highly predictable. However, subtraction of LookNeu condition probes controlled for probe anticipation effects. Moreover, the study may have been underpowered to show the effect of probe timing on startle inhibition. A post-hoc power analysis based on our results using G\*Power (Faul, Erdfelder, Lang, & Buchner, 2007) indicated that 262 participants would be necessary to achieve reasonable power (1-β>.80) in order to prove significance, given a true interaction effect. In our view, the cost to assess more data would outweigh the benefit to detect a potential but marginal effect. Finally, generalization of our results is limited because all participants were female college students. It has been shown that women respond with greater defensive reactivity to negative stimuli than men (Bradley et al., 2001), so effects observed in the present study might be less apparent in men.

**Conclusion**

Startle probes delivered at ≥7s are useful to quantify reappraisal effects, though earlier probes did not yield significantly worse effects. Moreover, the successful down-regulation of perceived arousal is reflected by a decline in the defensive tendency, measured with the emotion-modulated startle response. In contrast, down-regulation of emotional valence is not correlated with a reduction of the startle response.

**Footnotes**

**1** ECG data were also collected but not included in the present analyses.

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**Figure Captions**

*Figure 1.* Experimental procedure. Each trial began with a 2000-ms presentation of an instructional cue (view, down-regulate), followed by a fixation cross displayed for 1,000 ms. Next, a neutral or negative picture was presented for 12,500 ms. A startle probe (50 ms, 95dB white noise burst) was presented through headphones either at 2 s (probe A), 7s (probe B) or 12s (probe C) into the regulation phase. Probes were balanced across conditions and no more than two trials of same probe type were presented in consecutive trials. Self-assessment Manikins (SAM Ratings; Bradley & Lang, 1994) **were presented after picture offset.** Participants rated on a 1-9 Likert scale how positive/negative and aroused/calm they felt at that moment. Lower scores on the valence scale indicate that they felt more positive; lower scores on the arousal scale indicate that they felt calmer. By pressing buttons on a keyboard, subjects moved a bar from left to right to select SAMs corresponding to their subjective valence and arousal. The initial bar position was random and the final position of the bar at the end of the rating was logged. Valence and arousal rating scales were displayed consecutively for 5,000 ms each **with a 1,500 ms time lag between the ratings.** Intertrial intervals were jittered between 3,500 and 5,500 ms.

*Figure 2.* (a) Mean startle amplitudes in the RegNeg and LookNeg condition across the three startle probe times (A: 2s, B: 7s, C: 12s). Mean amplitudes represent T-score converted difference scores (RegNeg minus LookNeu and LookNeg minus LookNeu). Error bars represent standard errors of means (SEM). (b) Significant and positive Pearson’s correlation between arousal down- regulation and startle inhibition indicating that individuals who performed better at down-regulating arousal also performed better at inhibiting their startle response during reappraisal.