

Emotion modulation of the startle reflex during 300-ms picture presentation: a replication study

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Abstract

In the present study, the emotion modulation of the startle reflex after showing pictures of different emotional valence at varying probe times, was examined. The main objective was replicating Larson et al. (2005), who showed distinct patterns of emotion modulation at different probe times after a 300-ms presentation of pictures with different emotional content. By studying the emotion modulation of the startle with picture presentations of 300 ms, more information can be obtained about the usefulness of startle measurement in research on the immediate affective impact of pictures. In addition, a possible correlation between trait anxiety (STAI-score) and the mean blink magnitude was examined. This can give insight in the reactivity of individuals with high trait anxiety, which could be used in a clinical setting. Paired samples t-tests found no significant emotion modulation effect, nor did the correlation reach significance. This might indicate that a 300-ms picture presentation is not sufficient to evoke emotion modulation of the startle reflex or that the picture has to be present at the time of the probe in order to elicit emotion modulation.

Keywords: emotion modulation, startle reflex, trait anxiety, STAI, blink magnitude.

Introduction

Deficits in emotion regulatory abilities seem to be a core feature in several forms of psychopathology (Jackson, Malmstadt, Larson & Davidson, 2000). A physiological indication of emotion regulatory deficits is the startle reflex (Grillon & Baas, 2003), which has been shown to be increased in patients with anxiety disorders (Grillon et al., 2008) and people with high trait anxiety (Grillon, Ameli, Foot & Davis, 1993). This reflex is believed to be a defensive response, which can be evoked in an experimental context with, for example, the presentation of a loud noise blast. This startle reflex can be modulated by the emotional state of the participant, which can be influenced by the emotional content of pictures (Lang, Bradley & Cuthbert, 1990; Vrana, Spence & Lang, 1988).

A previous study on emotion modulation of the startle by Vrana and colleagues (1988), showed that the blink magnitude was augmented for unpleasant pictures and attenuated for pleasant pictures, both compared to neutral pictures. Therefore, the startle enables researchers to make distinctions between positive and negative states. However, Cuthbert, Bradley & Lang (1996) showed in a subsequent study that this effect was only elicited by high-arousing stimuli. If pictures were only moderately arousing, the emotion modulation effect was not significant.

Emotion modulation of the startle response is interesting to measure, because it can inform researchers about the mood and affective states of individuals (Bradley, Cuthbert & Lang, 1991; Vrana et al., 1988). The applicability of measuring emotion modulation of the startle reflex is not limited to healthy participants; its use can be expanded to the clinical population. By measuring emotion modulation of the startle in clinical and non-clinical populations, a possible clinical treatment could be more focused on these states and alteration of the way emotion is modulated.

Following Vrana et al. (1988), most studies presented emotional valenced stimuli with a duration of 6 seconds (Bradley, Lang & Cuthbert, 1993; Vanman, Boehmelt, Dawson & Schell, 1996). However, Vanman and colleagues (1996) showed that emotion modulation could already occur as early as 250 ms, which suggests that a 6-seconds-presentation of the stimuli is not necessary.

In contrast, a presentation of 30 ms did not produce the same emotion modulation effect as the previous studies. When emotional stimuli were presented for 30 ms, both negative and positive stimuli elicited a larger startle response compared to neutral stimuli. This suggests that briefly presented emotional stimuli access the

fast route of emotion recognition in which there can only be differentiated between emotional and non-emotional stimuli (Ruiz-Padial, Vila & Thayer, 2011).

Within the context of fast emotion modulation of the startle response, the study of Larson, Ruffalo, Nietert & Davidson (2005) is interesting. Not only did they use a condition in which stimuli were presented for 6 s, they also included a condition in which they presented stimuli for 300 ms. In both conditions, probes were presented at different times of 1.5 s, 4.5 s and 7.5 s after stimulus-onset. To measure stability of emotion modulation, 21 participants were tested twice in four weeks, which resulted in a total of 42 measurements. All these measurements were combined in z-transformed data with which t-tests were computed.

Larson et al. (2005) found that, in the 300 ms condition, pleasant and unpleasant pictures elicited significant larger blink magnitudes than neutral pictures after a probe time of 1.5 s, but there was no significant difference between pleasant and unpleasant pictures. In addition, attenuation of the startle response was significant when pleasant pictures were shown with a probe time of 4.5 s, compared to unpleasant and neutral pictures with a similar probe time. However, no significant results were found when the probe was presented 7.5 s after stimulus onset.

Replicating the 300-ms condition of Larson et al. (2005) will be the main objective of the present study. Replication will support the idea that startle measurement can be useful in research of the immediate affective impact of a stimulus. Based on the findings of Larson et al. (2005), we formulated our hypotheses and we expected to find the same results as them in the probe time conditions of interest, namely 1.5 s and 4.5 s after stimulus onset. However, a probe time of 7.5 s and trials in which no probe is audible will also be included to decrease predictability.

Additionally to the replication of Larson et al. (2005), another measure will be included in this experiment. Before viewing the pictures, participants will have to fill in the State/Trait Anxiety Inventory (STAI; Spielberger, 1983) to measure trait anxiety. Grillon et al. (1993) showed that participants with high trait anxiety showed a larger startle response than low trait anxiety participants, when they were threatened with an electric shock. We hypothesize that trait anxiety (STAI-score) and blink magnitudes, when viewing unpleasant and pleasant pictures, are positively correlated. If this correlation is significant, this may indicate that people with high trait anxiety react fast on arousing stimuli and interpret them as more threatening

(Klonowicz, 1987). This could indicate that an enhanced startle can be seen as a symptom of high trait anxiety. Testing the startle reflex could subsequently be used as a diagnostic tool. Moreover, measuring the startle reflex before and after treatment could indicate the effectiveness of the treatment (Grillon & Baas, 2003).

Methods

Power analysis

To determine the sample size of the present experiment, the standard error of the mean (SEM) and the standard deviations from Larson et al. (2005) were calculated. To replicate the significant findings of Larson et al. (2005), four independent tests had to be performed, which resulted in an alpha of .0125. In addition, a power of 0.8 was used. Calculations of four comparisons were inserted in G*Power (version 3.1), which resulted in four effect sizes:

1. Unpleasant compared to neutral pictures after a probe time of 1.5 s yielded an effect size of 0.99, considered to be extremely large using Cohen's d criteria.
2. Pleasant compared to neutral pictures after a probe time of 1.5 s resulted in an effect size of 0.67, considered to be large.
3. Unpleasant compared to pleasant pictures after a probe time of 4.5 s yielded an effect size of 0.91, considered to be extremely large.
4. Pleasant compared to neutral pictures after a probe time of 4.5 s resulted in an effect size of 1.07, considered to be extremely large.

To be able to detect an effect size of 0.67 with a power of 0.8 and an alpha of .0125, an a priori paired samples t-test was calculated which resulted in a minimal sample size of $N = 25$. However, in the original study of Larson et al. (2005), almost half of the participants were excluded due to technical difficulties or a lack of startle response. Therefore, we will use a sample size of $N = 25 + 11$, 36 participants.

Participants

Participants were 36 students (18 females and 18 males), which were from Utrecht University (between the ages of 18 and 25). Participants received experimental credit in exchange for participation. Participants with a history of psychological disorders, such as depression or anxiety disorders, were excluded from the experiment. These disorders may cause deviations in emotion modulation, which could manipulate the results. Three participants (3 males) were excluded from the analysis. One

participant fell asleep; another participant did not display any sufficient startles; and one had technical difficulties (the signal of an electrode was lost).

Materials

To replicate the research of Larson et al. (2005) as precise as possible, the same pictures from the International Affective Picture System (IAPS) were used. Larson and colleagues (2005) included these specific pictures, because they were rated as equally arousing by the participants. A total of 63 pictures, 21 of each valence, were presented in a counterbalanced order across participants. In addition, 12 extra pictures of neutral valence were included to utilize in the habituation phase. These pictures were selected additionally to the pictures used in Larson et al. (2005) and can be found in Appendices A and B.

Procedure

Upon arrival participants were informed about the procedure of the experiment. After signing the informed consent, two electrodes were placed on the face. At first, participants completed a computerized Dutch version of the STAI. Then, the experiment started with a habituation phase in which participants viewed 12 neutral pictures, with a random interval between 10 and 18 seconds, and were probed with a loud noise through their headphones. After the habituation phase, the actual experiment started.

Participants viewed pictures at one single session. During this session, probes occurred during six trials of each valence, for all of the three probe times. In addition, in three trials of each valence no probe was presented. All these conditions are displayed in table 1. The duration of the acoustic startle probe was 50 ms with 100 dB white noise burst. Pictures were presented for 300 ms with a randomized 10- to 18-s intertrial interval (mean ITI = 14 s). Therefore, all probes occurred after picture offset. No instruction was given to the participants to maintain an image of the picture after its offset.

Table 1

The different conditions used in the experiment.

Condition	Valence	Probe time
NeuE	Neutral	1.5 s
NeuM	Neutral	4.5 s
NeuL	Neutral	7.5 s
PosE	Positive	1.5 s
PosM	Positive	4.5 s
PosL	Positive	7.5 s
NegE	Negative	1.5 s
NegM	Negative	4.5 s
NegL	Negative	7.5 s

Startle Recording and Quantification

In this study, the same methodology as Klumpers et al. (2010) was used. Following these authors, raw and integrated EMG data were collected by placing two electrodes on the orbicularis oculi, located directly below the left eye and administered ± 15 mm apart. The blink magnitudes were recorded and amplified using a Biosemi Active Two System, with Ag-AgCl electrodes at a sampling frequency of 2 kHz. The initial EMG signals were filtered using a 28-Hz, 12-dB/oct high-pass and a 500-Hz, 24-dB/oct low-pass filter. Additionally, the data were segmented into epochs, which started 50 ms before the onset of the startle probe and ended 200 ms after onset. Lastly, the signal was baseline corrected and rectified, and a low-pass filter of 14 Hz, 24 dB/oct was applied for smoothing.

After filtering the raw data, an artifact rejection procedure was performed on the raw and unfiltered data. Trials with a baseline (-30 to 20 ms) activity greater than 2 standard deviations from the mean baseline activity were rejected. Eye-blinks in which excessive noise was present during a 50 ms prestartle, baseline period were excluded in analyses. The same procedure holds for integrated eyeblink reflexes of which the onset is less than 90 ms or more than 150 ms following the probe. Furthermore, blinks greater than 3 standard deviations above the mean for each participant were excluded. In addition, participants of whom more than half of the

trials were bad, were excluded from the analyses. Lastly, analyses were restricted to participants with at least three good responses in every condition.

The mean blink magnitude for each condition was computed for each participant and z-transformed. Four two-tailed, paired samples t-tests were calculated to compare the valence with blink magnitude at a certain probe time. The first two t-tests compared condition 1 to conditions 4 and 7. We expected that the mean blink magnitude in conditions 4 and 7 was higher than in condition 1. The third and fourth t-tests compared condition 5 to conditions 2 and 8. The mean blink magnitude in condition 5 was expected to be lower than in conditions 2 and 8. In addition, a correlation was calculated to determine the degree of correlation between trait anxiety and the magnitude of eye blink after viewing negative and positive pictures. We expected the mean blink magnitude to be positively correlated with both negative and positive picture presentations.

Results

Emotion Modulation Effects

Standardized data. Four two-tailed, paired samples t-tests with an alpha level of .0125 were conducted and revealed no statistically significant effect. The standardized data is displayed in figure 1. NeuE (M = -0.130, SD = 0.423) versus PosE (M = -0.120, SD = 0.378), $t(32) = -0.133$, $p = .895$, $d = 0.011$. NeuE versus NegE (M = 0.030, SD = 0.436), $t(32) = -1.48$, $p = .149$, $d = 0.12$. NeuM (M = -0.070, SD = 0.343) versus PosM (M = -0.056, SD = 0.371), $t(32) = -0.157$, $p = .876$, $d = 0.081$. PosM versus NegM (M = 0.010, SD = 0.360), $t(32) = 0.676$, $p = .504$, $d = 0.0052$.

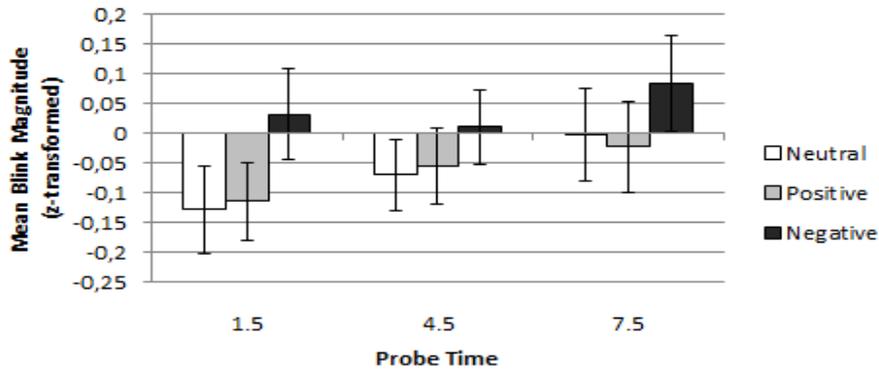


Figure 1. Standardized (z-score) blink magnitude averaged across assessment for each probe time and picture content.

Raw data. In figure 2, the raw data is displayed. As can be seen, the same pattern is found as in the standardized data. To measure if the startles were accurately measured, six one-sample t-tests were conducted in comparison with 0. All six t-tests revealed significant results. NeuE (M = 42,63, SD = 41,32), $t(32) = 5,927$, $p = .000$, $d = 1.03$. PosE (M = 42,62, SD = 37,98), $t(32) = 6,446$, $p = .000$, $d = 1.11$. NegE (M = 46,10, SD = 36,73), $t(32) = 7,211$, $p = .000$, $d = 1.12$. NeuM (M = 43,91, SD = 39,79), $t(32) = 6,34$, $p = .000$, $d = 1.05$. PosM (M = 45,28, SD = 42,93), $t(32) = 6,059$, $p = .000$, $d = 1.26$. NegM (M = 45,22, SD = 41,83), $t(32) = 6,210$, $p = .000$, $d = 1.08$.

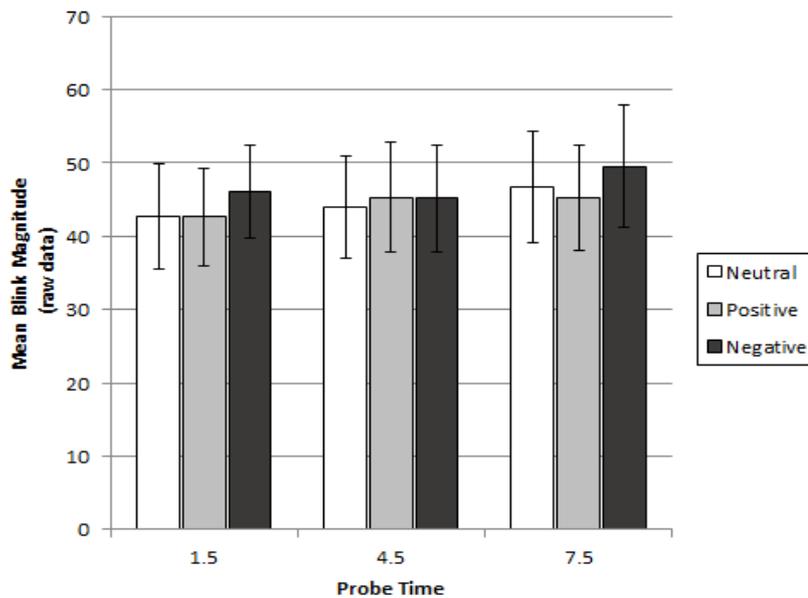


Figure 2. Raw data blink magnitude averaged across assessment for each probe time and picture content.

Correlation STAI-score and blink magnitude

Standardized data. Two bivariate correlations were used to assess the linear relationship between the STAI-score and the blink magnitude in two valence conditions, PosE and PosM combined, and NegE and NegM combined. The correlation between the STAI-score and the positive pictures (figure 3), was statistically non-significant, $r(31) = -.03$, $p = .852$. In addition, the correlation between the STAI-score and the negative pictures (figure 4), did not result in a significant result either, $r(31) = .06$, $p = .753$.

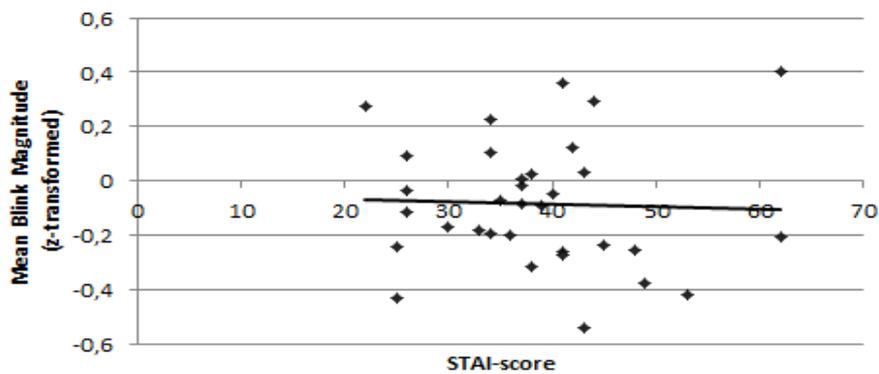


Figure 3. Correlation of standardized (z-score) blink magnitude averaged across assessment for each probe time and positive picture content, and the STAI-score.

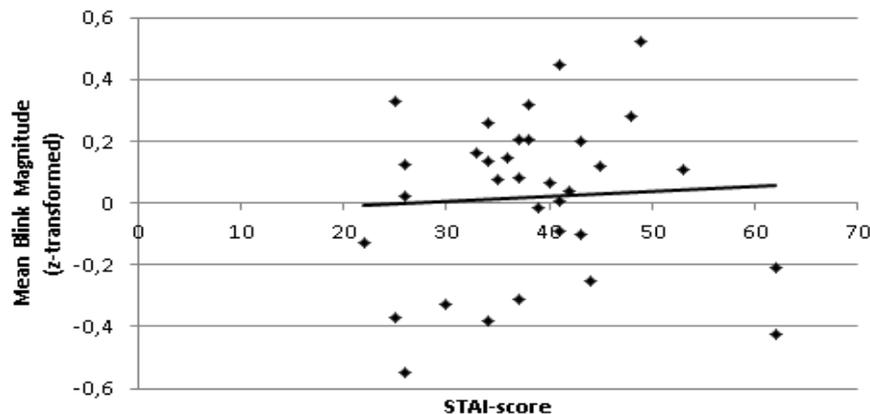


Figure 4. Correlation of standardized (z-score) blink magnitude averaged across assessment for each probe time and negative picture content, and the STAI-score.

Raw data. Two identical bivariate correlations computed for the raw data also revealed a non-significant statistical result. The pattern seen in the standardized data of positive pictures, reveals a slightly descending average blink magnitude when the STAI-score is higher. The raw data in figure 5 reveal a slightly different pattern,

where the mean blink magnitude does increase when the STAI-score is higher. However, this correlation is not significant, $r(31) = .12, p = .494$.

For the correlation between negative pictures and the STAI-score, both the standardized data and the raw data show the same pattern of results. In both correlations (figures 4 and 6), a slightly ascending trend is visible. The correlation computed for the raw data did not reveal a significant effect, $r(31) = .15, p = .402$.

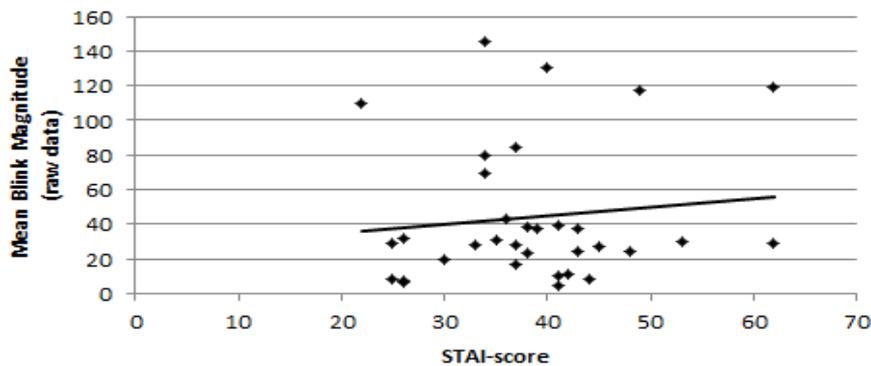


Figure 5. Correlation of raw data blink magnitude averaged across assessment for each probe time and positive picture content, and the STAI-score.

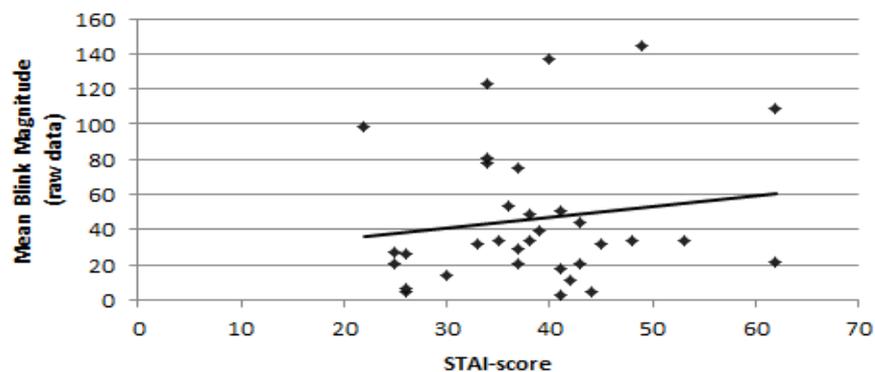


Figure 6. Correlation of raw data blink magnitude averaged across assessment for each probe time and negative picture content, and the STAI-score.

Discussion

The data of the present study do not support the claim of Larson et al. (2005), since emotion modulation of the startle after a 300-ms presentation of pictures was not statistically significant. Both pleasant and unpleasant pictures, followed by a probe after 1.5 s, did not result in significant potentiation of the blink magnitude compared to neutral pictures. Moreover, pleasant pictures followed by a probe time of 4.5 s, did

not result in significant attenuation of the blink magnitude compared to both unpleasant and neutral pictures. A similar pattern has been found in the raw data.

A possible explanation for the incongruence between the results of Larson et al. (2005) and the present study, could be that the startles were not well enough recorded. However, the raw data indicate that the startles from the present study were well-recorded. Six one sample t-tests all revealed significant differences between the peak amplitude of the mean startles and a zero-baseline.

The fact that no significant effects were found in the present study may also be explained by the relative small size of the sample. While Larson et al. (2005) used a dataset of 42 measurements (21 participants tested twice), we only had 33 measurements in our dataset (33 participants tested once). The sample size of the present study was based on a power analysis, which was computed with the effect sizes of Larson et al. (2005). However, the effect sizes that Larson et al. (2005) found were considered to be large or extremely large. As a result, the calculated and required sample size of the current study was relatively small. With a larger sample size or dataset, a significant effect may have been obtained. For future studies, we suggest using a larger sample size, and, if possible, a power analyses based on the effect sizes of multiple studies.

The current study also shows the importance of replication studies in social sciences, since large effect sizes in one study, cannot always be replicated in other studies. Studies with large effect sizes could have large implications, since a large effect size would, in this case, suggest that the startle is a useful method to measure the immediate affective impact of a picture. However, the present study shows that this assumption should be questioned and that startle research should be reviewed critically.

The data from the present study also show that the correlation between the STAI-score and startle magnitude did not reach significance. Therefore, our findings do not support Grillon et al. (1993), who showed that participants with high trait anxiety show a larger startle response than participants with low trait anxiety. This inconsistency may be explained by the fact that Grillon et al. (1993) used another type of aversive stimuli. Unpleasant pictures may be experienced as less aversive than electric shocks. Since the correlation was not significant, the measurement of the startle as a diagnostic tool for high trait anxiety may not be useful.

Moreover, the present study does not confirm the claim of Bradley et al. (1991) and Vrana et al. (1988) that emotion modulation of startle response can inform researchers about the mood and affective states of individuals. However, it needs to be mentioned that both Bradley et al. (1991) and Vrana et al. (1988) used a picture presentation of 6 s, while the present study used a picture presentation of 300 ms. Therefore, it is possible that only a 6 s presentation can inform researchers about the mood and affective states of individuals. In conclusion, we agree with Larson et al. (2005) that more research needs to be done on the relationship between individual differences in emotion modulation of the startle and differences in their mood and affective reactivity.

At last, two critical points on the present study have to be mentioned. First of all, most of the participants were highly educated students. Therefore, the sampling of the study was not completely random. Second of all, participants with clinical diagnosis of anxiety were excluded from the study. However, it is possible that a correlation between blink magnitudes and the STAI-score is only present when people with a clinical diagnosis of anxiety was compared to the non-clinical population. Therefore, we suggest that further research may include a clinical population as well as a non-clinical population and also include participants with different education levels.

The incongruence in results between the present study and the study of Larson et al. (2005) is interesting for further research in order to test if a 300-ms presentation of pictures is enough to elicit emotion modulation of the startle reflex. The results suggest that a longer duration of the stimuli is necessary. One possible explanation for this may be that stimuli need to be present at the time of the probe to evoke emotion modulation of the startle reflex. Another explanation could be that more time is needed for the participant to process the stimuli. Several articles have shown emotion modulation after a picture presentation of 6 s, but it would be interesting to study how long a picture needs to be displayed to elicit emotion modulation. Therefore, a question for further research could be whether a picture presentation of, for example, 1 s or 3 s is long enough to elicit emotion modulation.

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Appendices

Appendix A: Picture Set A

Pleasant Pictures	Description	Number	Gender	Unpleasant Pictures	Description	Number	Neutral Pictures	Description	Number	Habituation phase
leopard	by water	1650		boy	licking cow	2730	male	tapped	2190	
couple		4608		mafia	hit	3010	man	with hat	2570	
couple		4680		burn	victim	3053	person's	shadow	2880	
astronaut		5460		throat	lash	3071	layered	mushrooms	5510	
space shuttle		5450		burn	victim	3100	mushrooms	in lover	5530	
windsurfers		5623		burn	victim	3110	three	dry mushrooms	5534	
hiker	along bridge	5629		mutilated	body	3130	green	plant and soil	5740	
fireworks		5910		baby	with eye tumor	3170	rolling	pin	7000	
chocolate	drink	7270		subway	robbery	3500	spoon		7004	
ski jump	90M	8030		aimed	gun	6230	blue	mug	7009	
extended	sailor	8080		aimed	pistol	6260	fan		7020	
sailing		8170		knife	assault at door	6313	claw	hammer	7034	
powder	skiing	8190		man	slapping wife	6360	hair	dryer	7050	
catamaran		8210		man	with ski mask	6510	old	book	7090	
shooting	rapids	8370		knife	assault	6550	painted	fabric	7160	
rafting	duo	8400		suicide		6570	square	block	7185	
roller	coaster	8490		plane	evacuation	9050	fabric	and beads	7207	
US	currency	8501		torture	& beating	9252	decorated	plate	7233	
male	with groceries	4532	female	decaying	animal	9570	window	& blue wall	7490	
couple	on street	4599	female	male	neo-Nazi	9800	unmade	bed with white	7710	
couple	gazing	4609	female	auto	wreck	9910	block	boy pondering	9070	
couple	sex	4607	male				farmer	on field	2191	yes
couple	nude	4664	male				shoes		7031	yes
motorcycle	racing	8260	male				industrial	harbor	7036	yes
							dustpan		7040	yes
							weights		7042	yes
							clothespins		7052	yes
							coffee	and newspaper	7057	yes
							truck		7130	yes
							bus		7140	yes
							table	with pastries	7220	yes
							blue	and yellow balls	7238	yes
							laundry	at balconies	7242	yes

Appendix B: Picture Set B

Pleasant Pictures	Description	Number	Gender	Unpleasant Pictures	Description	Number	Neutral Pictures	Description	Number	Habituation phase
three	puppies	1710		mutilated	face	3000	neutral	male, young	2200	
couple	kissing	4660		face	laceration	3030	itnerant	Latino boy	2870	
couple	nude	4690		mangled	face	3060	two	people	2890	
astronaut	& earth	5470		missing	face	3080	three	mushrooms	5520	
skydivers	in a circle	5621		burn	victim, left chest	3102	mushroom		5531	
hang-glider		5626		mutilated	body	3120	blue	door	5731	
rocky	mountain peak	5700		mutilated	hand	3150	electric	outlet	6150	
turkey	dinner	7230		severed	hand	3400	blue	towel	7002	
Disney	castle	7502		man	with gun in mouth	3530	white	bowl	7006	
slalom	ski racer	8034		aimed	pistol	6250	wicker	basket	7010	
gymnast		8090		man	grabbing woman	6312	wooden	stool	7025	
cliff	divers	8180		knife	assault	6350	glass	mug	7035	
water	skier	8200		man	with ski mask	6370	fork,	silverware	7080	
pilot	& prop plane	8300		knife	assault	6540	yellow	fire hydrant	7100	
victorious	relay team	8380		man	holding gun	6560	brass	and wood lamp	7175	
victorious	gymnast	8470		gang	attacking car	6821	scarves,	etc., pastel	7205	
gold	bars	8500		medics	carrying victim	9250	clothes	rack	7217	
stack	of money	8502		man	with head of child	9410	wooden	chair	7235	
male	volleyball	4533	female	sinking	ship w/ nosedive!	9600	lab	building	7491	
fireman	nice chest	4572	female	Klansmen	& cross	9810	box	of Kleenex	7950	
couple	kissing	4640	female	firefighters		9921	environmental	workers	9700	
couple		4652	male				farmer	on field	2191	yes
smiling	female	2030	male				shoes		7031	yes
pilot	towing hang-glider	8340	male				industrial	harbor	7036	yes
							dustpan		7040	yes
							weights		7042	yes
							clothespins		7052	yes
							coffee	and newspaper	7057	yes
							truck		7130	yes
							bus		7140	yes
							table	with pastries	7220	yes
							blue	and yellow balls	7238	yes
							laundry	at balconies	7242	yes