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The association between attentional bias and fear potentiated startle
in an emotional cuing- and fear-potentiated startle paradigm

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Abstract

Recent studies show that all individuals show attentional biases to threat when presented with an unambiguous or highly threatening situation. However, it is still unknown if these biases are related to an oversensitive threat detection system that high trait anxious individuals are thought to possess. To test this hypothesis, this study examined the relationship between attentional biases to threat, fear-potentiated startle and skin conductance responses. An unselected sample of participants (n=30) completed an emotional cuing task and a startle- and skin conductance procedure in which they were presented with neutral face stimuli. One of the faces (threat cue) was followed by an aversive noise while the other was not (neutral cue). Startle- and skin conductance responses were then measured. Participants reacted faster to valid trials than to invalid trials on the emotional cuing task. However, they did not differ in the speed of their responses on either neutral or threatening trials. Contrary to expectation, skin conductance responses were not related to any of the measures, while fear potentiated startle only showed a negative relation with engagement scores. Implications are discussed.

Keywords: attentional bias, emotional cuing, fear-potentiated startle, trait anxiety,

The ability to automatically pay attention to potential threats in the environment is highly adaptive. It allows us to successfully deal with threatening situations by rapid detection of important information, while less important information disappears to the background (Bar-Haim et al., 2007; Öhman, Flykt, & Lundqvist, 2000). When a person encounters an aversive or secondary aversive stimulus, for example when a conditioned stimulus is associated with punishment, the behavioural inhibition system (BIS) is activated. The person is likely to react to the stimulus by stopping current actions and searching the environment for threat. The behavioural activation system (BAS) on the other hand, is reactive to stimuli of reward and promotes approaching behaviour and positive emotions (Gray and McNaughton, 2000). Findings from behavioural studies suggest that threatening stimuli automatically capture attention. This effect is thought to be mediated by a subcortical brain network centered on the amygdala (Öhman, 2005). Because of its importance to survival, this automatic attentional mechanism is present in all of us. However, people differ in their tendency to perceive a situation as threatening or safe. The level of reactivity of the BIS is associated with trait anxiety (Heubeck et al., 1998). A host of studies has shown that individuals with high levels of trait anxiety are prone to a selective attentional bias that leads them to attend more to threat-related stimuli than low trait anxious (LTA) individuals (Cisler & Koster, 2010; Eysenck, 1997; Eysenck, 2000; Koster et al., 2004; Koster et al., 2006; Mogg and Bradley, 1998; Mathews, & MacLeod, 1996).

Attentional biases reflect the way individuals allocate their attention toward threatening stimuli relative to neutral stimuli. Three influential paradigms that have documented attentional biases are the emotional Stroop, dot probe, and emotional spatial cuing (Bar-Haim et al., 2007). In the emotional Stroop task, participants are presented with words printed in different colours and are required to name the colour as fast as possible without paying attention to the meaning of the word. Numerous studies have found that high trait anxious

(HTA) individuals tend to focus attention on task-irrelevant emotional aspects (e.g., threatening content of the words) of the task (Bar-Haim et al., 2007, Williams et al., 1996, Reinholdt-Dunne et al., 2009). However, the emotional Stroop task has been criticized for lacking in validity, urging MacLeod et al. (1986) to develop the dot-probe paradigm. In this task, participants are presented simultaneously with one threatening and one neutral stimulus on each trial, followed by a small target probe in the locations where the stimuli were just presented. Participants are asked to respond as fast as possible to the target probe. MacLeod, Mathews, and Tata (1986) showed that patients with generalized anxiety disorder (GAD) were faster to detect a target probe when it appeared in the location where recently a threat-related word had appeared, compared to normal control participants. Faster responses to target probes that replace threat-related as opposed to neutral stimuli are thought to indicate an attentional bias. Contrary to the dot-probe task, in the emotional spatial cuing task, only one cue is presented at a time. The cue appears in one of two locations and is followed by the target. The faster reaction to targets appearing in the location where the cue has appeared just before (validly cued target) relative to targets appearing in the opposite location (invalidly cued targets) is called the cue validity effect (Posner, Inhoff, Friedrich, & Cohen, 1987). A threat-related bias occurs when validity effects are larger when the cue is threatening rather than neutral (Bar-haim et al., 2007). An important advantage of spatial cuing tasks is that they enable us to differentiate between the components of attentional bias (Cisler & Koster, 2010) and investigate whether attentional biases are due to faster engagement to relevant threat cues or difficulty disengaging from irrelevant threat cues (Bar-Haim et al., 2007). Facilitated attention is indicated by faster responses on valid threat cued trials relative to valid neutral-cued trials. It has been proposed to be the result of a largely automatic threat detection mechanism. Difficulty in disengagement may be a mixture of automatic and strategic

processing and is reflected by reaction time costs in responding to invalid threat-cued trials relative to invalid neutral-cued trials (Cisler & Koster, 2010).

The evidence for facilitated attention to threat cues among anxious individuals is mixed. Several studies employing spatial cuing tasks have shown that when neutral stimuli are made to signal an aversive event, they are able to induce facilitated attention (Koster, Crombez, Van Damme, et al., 2004; Van Damme et al., 2006). Other studies have not found faster attentional engagement (Fox, Russo, & Dutton, 2002; Yiend & Mathews, 2001), possibly because facilitated attention is moderated by threat intensity and stimulus duration. In order to effectively induce facilitated attention, it has been suggested that stimuli should be sufficiently threatening and have a duration of 100 ms or less (Cisler & Koster, 2010). For instance, a study by Koster, Crombez, Verschuere, Van Damme, et al. (2006) showed that facilitated attention occurred after presenting high trait anxious participants with highly, but not mildly, threatening pictures at 100 ms stimulus durations. Longer presentation durations of the cues did not result in facilitated attention. Studies using the spatial cuing task have also shown that anxious individuals have difficulty in disengagement (Cisler & Olatunji, 2010; Fox, Russo, & Dutton, 2002; Koster, Crombez, Verschuere, Van Damme, et al., 2006; Van Damme, Crombez, Hermans, Koster, & Eccleston, 2006; Yiend & Mathews, 2001). Recently, Massar and colleagues (2010) showed that disengagement effects could be induced in both high- and low anxious participants. In a follow-up study using groups of preselected HTA and LTA participants, they replicated these findings, reporting impaired disengagement for both groups and facilitated engagement for HTA participants.

In emotional spatial cuing paradigms, the repeated presentation of a neutral stimulus coupled to a threatening event results in both attention towards the conditioned stimulus (Van Damme, Crombez, Hermans, Koster, & Eccleston, 2006) and anticipatory fear that facilitates motor reflexes. The amygdala is thought to play an essential role in the establishment of the

association between the neutral stimulus and the following aversive event. A number of functional imaging studies has shown increased activation of the amygdala to conditioned aversive stimuli in humans (Büchel et al., 1999; Morris et al., 2001; Critchley et al., 2002; Phelps et al., 2004). However, several other studies have not found learning-related amygdala activation in conditioning paradigms (Cheng et al., 2003; Knight et al., 1999; Knight et al., 2004; Fischer et al., 2002). An often used physiological measure of anxiety is the fear-potentiated startle reflex (Davis 1986; Grillon and Baas 2003). Like attentional biases, the startle reflex is a rapid reaction to a threatening event and is important for adaptation to the environment (Li and Yeomans, 1999; Li, Steidl, & Yeomans, 2001). When an individual is startled by a sudden event, the orbicularis oculi muscle will rapidly contract. This vigorous blink of the eye has been named the startle reflex. The strength of the startle reflex varies with the individual's emotional state (Lang, Bradley, & Cuthbert, 1990; Vrana, Spence, & Lang, 1988; Waters, Lipp, & Spence, 2005). When presented with a sudden aversive stimulus, a person who is already on edge is likely to show a more vigorous eyeblink than a person who is at ease. In an experimental procedure used to elicit the startle response, participants can be trained to fear a neutral stimulus when it is followed by an aversive event (e.g., an acoustic stimulus). This stimulus then signals to the participant that the aversive event will shortly occur (Lang et al., 2000). Even if the threatening event does not take place, the mere verbal threat is sufficient to increase blink magnitude (Grillon et al., 1991). The acoustic stimulus used to elicit startle can be quite small (usually a 50 ms burst of white noise at around 95 dB) and yet cause a clear startle response which typically does not interfere with the experimental tasks (Lang, Davis, & Öhman, 2000). When the central nucleus of the amygdala has been damaged and either a visual (Hitchcock and Davis, 1986) or auditory conditioned stimulus (Hitchcock and Davis, 1987; Campeau and Davis, 1995) is used, the fear-potentiated startle is blocked. Individuals vary in the arousal that they experience while being fearful. Autonomic

arousal can be indexed by skin conductance responses (SCR). SCR reflects electrodermal changes associated with activity of the eccrine sweat glands on the palms of the hands (Tremayne & Barry, 2001). SCR is often used to measure fear-induced arousal, for instance measured in groups of participants with a specific phobia (Globisch et al., 1999; Öhman, 2005; Öhman & Soares, 1994).

An interesting question is whether attentional biases that are induced in healthy participants when they are faced with an unambiguously threatening situation are associated with an oversensitive threat detection system, caused by an overactivation of the amygdala. If this is the case, it can be expected that an individual's magnitude of the fear potentiated startle is positively associated with the size of their attentional biases. To my knowledge, no studies have as yet tried to find an association between individuals' attentional bias to threat and the magnitude of their startle response and skin conductance response. To this end, the first of the two experimental procedures used by Massar and colleagues (2010) is replicated with the addition of an experimental procedure measuring startle- and skin conductance responses.

Several hypotheses regarding the effect of attentional bias on participants' responses are tested. Firstly, I hypothesize that participants in an unselected sample will show a cue validity effect in the emotional cuing task. That is, they will be faster to respond to valid cued trials rather than to invalid cued trials. Secondly, I hypothesize that participants will be faster to respond to valid threatening trials compared to neutral trials and slower to invalid threatening trials compared to invalid neutral trials. Thirdly, I hypothesize that these attentional biases are associated with participants' emotional responses to aversive stimuli (startle- and SCR-effect). Furthermore, I hypothesize that participants' self-reported level of trait anxiety will be positively related to the size of the engagement- and disengagement effect, and to the magnitude of their startle- and skin conductance response. I further hypothesize that participants' self-reported levels of BIS will be positively related to the size of the

engagement- and disengagement effect and to the magnitude of startle- and skin conductance responses, whereas self-reported levels of BAS will show a negative relation. Previous research has found a robust relationship between trait anxiety and worry (Constans, 2001; Davey, 1994; Wells, 1994). Trait anxiety scores are therefore expected to be positively associated with worry scores, as measured by the Penn State Worry Questionnaire (Meyer, Miller, Metzger, & Borkovec, 1990). Finally, self-reported scores on the attentional control scale by Derryberry and Reed (2002) are expected to be positively associated to trait anxiety and impaired disengagement, because low attentional control has been linked to both impaired disengagement from threat (Derryberry & Reed, 2002; Peers and Lawrence, 2009) and trait anxiety (Bishop, 2008; Derryberry & Reed, 2002).

Methods

Participants

This experiment was part of a larger study, which included the administration of electric shocks in the procedure directly following this experiment. An unselected sample of 35 participants was recruited (18 males, 17 females; mean age (s.d.) = 23.5 (3.6)) by flyers handed out to students at the Utrecht University campus. For their participation, they received either course credits or €8,-. All participants provided their written informed consent before the experiment and were told that they were free to terminate the experiment at any time.

Equipment

Emotional Cuing Task

The visual stimuli were presented on a CRT computer monitor (refresh rate: 75 Hz) on an IBM-compatible computer using the E-prime 1.1 (SP3) software package (Psychological Software Tools). A pair of Sennheiser headphones was used to binaurally present sounds.

Physiological apparatus

The eyeblink component of the startle response was measured by recording the activity from the orbicularis oculi muscle beneath the right eye using Active Two electrodes (BioSemi). Startle was elicited by a 50-ms duration, 100dB(A) burst of white-noise presented binaurally through a pair of Sennheiser headphones.

Two Galvanic Skin Response (GSR) electrodes were put on of the fore finger and the ring finger of the left hand to measure skin conductance responses. The activity of the electromyogram (EMG) and skin conductance response (SCR) was recorded using the data acquisition program Actiview (BioSemi).

Emotional cuing task

The emotional cuing task (adapted from Koster et al., 2004) was a counterbalanced within-subjects design in which participants were conditioned to associate a neutral face cue with an aversive noise (see Figure 1). While focusing on a white fixation cross ($1 \times 1^\circ$ visual angle) in the center of a black screen, one of two face cues (pictures of male faces in either a blue or yellow colored box) was presented to participants for 200 ms in a rectangles outlined in white (height 6.5° , width 4.8°) either to the left or right of the fixation cross. The distance from the centers of the boxes to the center of the screen was 9.2° . After 13.3 ms, a black $1 \times 1^\circ$ square (the target probe), was presented in either one of the boxes. In valid trials (75%), the cue was followed by a target probe in the same box. In invalid trials (25%), the target probe appeared in the box opposite to the cue. Participants were asked to press 'q' on the keyboard if the dot appeared in the left box, and 'p' if it appeared in the right box. Catch trials were included to test whether participants were really responding to the target probes, rather than to the face cues. During catch trials, no target stimulus was presented and participants were required not to press any key. The fixation cross was sometimes briefly (100 ms) replaced with a digit (0.5° in height), which had to be named aloud as fast as possible by the participant. These

digit trials ensured that participants would focus their gaze on the fixation cross, rather than on one of the face cues. To acquaint participants with the task, they started with a baseline task, in which face cues, but no auditory stimuli were presented. The baseline task was followed by the second task started, in which one of the face cues was linked to an aversive noise (the treat cue), and the other was linked to a neutral sound (the neutral cue). Participants were explicitly instructed which face cue was coupled to the aversive noise. The color and identity of the threat and the neutral face cues was counterbalanced between subjects. Sounds were delivered 200 ms after participants responded, in 25% of trials. The practice task and the test phase consisted of 72 valid trials, 24 invalid trials, 6 catch trials and 6 digit trials. The aversive noise was a recording of a human scream adapted from previous work by Lissek et al. (2005, duration 300 ms; 100 dB(A)), while the neutral sound cue was a 1000 Hz sine wave (300 ms; 70 dB(A)).

Startle and Skin conductance measurements

Startle response (SR) and skin conductance response (SCR) were measured in a physiological procedure following the emotional cuing task. Once again, participants first engaged in a baseline procedure before proceeding to the second procedure. In order to reduce initial startle reactivity, the procedure started with a startle habituation sequence, consisting of three startle sounds (Inter trial interval (ITI) = 8 – 11 s), and one silence sound (ITI = 3 s). Participants were required to sit still and merely focus on the fixation cross. During the baseline procedure, participants were presented with the same face cues as in the emotional cuing task. Startle sounds were only played after presentation of the aversive face cue. The baseline procedure consisted of 24 trials with a counterbalanced presentation of cues to the left and right. Eight trials were used to measure SCR (four threat and four neutral) and 16 trials measured startle response (eight threat and eight neutral). Before the start of the second physiological procedure, participants were again subjected to a startle habituation sequence of

three startle sounds and one silence sound. The second procedure consisted of 26 trials. Four trials were reinforcement trials (two for threat and two for neutral) of which two (one for threat and one for neutral) were combined with a startle trial. One trial lasted 1500 ms. A trial started with the presentation of one of the face cues (1100 ms), followed by a 50 ms presentation of the face cue which was sometimes accompanied by a startle noise. Then, another 50 ms presentation of only the face cue, followed by a 300 ms presentation of the face cue which was occasionally accompanied by a reinforcement during the second procedure. Finally, a background picture was shown, for which different ITI's were used: 12 s after startle sounds and reinforcements, and 8 s after the sole presentation of a face cue used to measure SCR.

Questionnaires

Participants were presented with four questionnaires after completion of the experiment. They filled in Spielbergers State-Trait Anxiety Inventory (STAI) (Spielberger, Gorush, & Lushene, 1970; van der Ploeg, Defares, & Spielberger, 1980), The Penn State Worry Questionnaire (Meyer, Miller, Metzger, & Borkovec, 1990), The BIS/BAS questionnaire (Carver & White, 1984) and the Attentional Control Scale (ACS) (Derryberry & Reed, 2002; translated in Dutch by M. Morren).

Procedure

Upon arrival at the testing lab, participants filled in the consent form and were seated behind a desktop computer. The electrodes were attached under participants' right eye, on their forehead and on their left hand. The lights were then turned down and the emotional spatial cuing task was started. To acquaint participants with the task, they first performed a practice task. After completion, participants were informed by a text on the computer screen that their physiological reactions would be measured in the upcoming part of the experiment

and that loud noises would occasionally be played through the headphones when either the threatening or neutral face cue appeared. They were instructed to fix their gaze on the fixation cross in the center of the screen while sitting still, in order to obtain clear physiological measurements. They were then informed that this part of the experiment would start with three loud noises that might startle them. The habituation sequence started, followed by the baseline physiological procedure. After completion, participants moved on to the second cuing task. Participants were explicitly informed which cue would be followed by the aversive sound. This was a replication of the baseline cuing task, with the exception that now, one face cue was followed by an aversive sound, and the other was followed by a neutral sound. The second cuing task was followed by the second physiological procedure. After completion, a manipulation check was carried out in which participants were asked how (un)comfortable they felt viewing the two face cues on a 10-point Likert scale (0 = very comfortable; 9 = very uncomfortable) and how strongly they expected a scream to follow the cues (0 = certainly not, 9 = certainly). They also rated the aversiveness of the scream (0 = absolutely not aversive, 9 = highly aversive). Finally, they filled in the four questionnaires. The light was turned back on and the researcher entered the test lab to debrief the participant.

Data reduction and analysis

The EMG signals were analyzed with Brain Vision Analyzer software. The strength of the startle reflex was defined as the peak of the startle response in the period of 20 to 120 ms after presentation of the aversive stimulus. The magnitude of the skin conductance responses was defined as the peak between 1000 to 6000 ms after stimulus presentation. The startle effect was determined by subtracting the threatening baseline startle from the threatening acquisition startle, minus subtracting the neutral baseline startle from the neutral acquisition startle [(threat acquisition - threat baseline) – (neutral acquisition – neutral baseline)]. The skin conductance response effect (SCR-effect) was computed in the same way [(threat acquisition-

threat baseline) – (neutral acquisition – neutral baseline)]. Four participants showed no skin conductance during half of the trials or more and were removed from the sample. For the emotional cuing task, reaction time data were cleaned by removing errors. Reaction times faster than 150 ms, or slower than 1000 ms were considered outliers, and were not included in the analysis (Koster et al., 2004; Massar et al., 2010). One participant was removed from the sample because of missing data in the cuing task. Baseline emotional cuing reaction times were transformed to median emotional cuing reaction times. In order to test for an effect of threat on reaction times, a 2 x 2 (Validity: valid, invalid; Cue type: threat, safe) repeated measures ANOVA was carried out using median emotional cuing reaction times. The total threat cuing effect was computed by subtracting the validity effect for the threatening cue from the validity effect for the neutral cue: $RT(\text{validity effect neutral cue}) - (\text{validity effect threatening cue})$. Attentional engagement was determined by subtracting reaction times of valid threat cues from valid neutral cues: $RT(\text{valid neutral cue}) - RT(\text{valid threat cue})$. Attentional disengagement was computed by subtracting the reaction times of invalid neutral cues from invalid threatening cues: $RT(\text{invalid threat cue}) - RT(\text{invalid neutral cue})$. In these computations, larger positive values represent stronger modulation. A bivariate correlational analysis was performed to check for associations between the scores on the questionnaires, the threat-cuing effect the startle-effect, the SCR-effect and engagement- and disengagement scores.

Results

The sample (N=30) consisted of 16 males and 14 females with a mean age of 23.7 (s.d. = 3.8). The questionnaires had the following mean (s.d.) scores: 37.5 (8.9) for STAI, 20.3 (4.0) for BIS, 17.2 (2.0) for BAS, 49.6 (6.9) for ACS and 44.5 (12.0) for PSWQ.

Subjective Ratings

Participants rated the aversiveness of the noise on a 10-point Likert scale (0 = not at all bothersome, 9 = very bothersome) as 7.4 (1.9). Although they rated the likelihood that an aversive sound would be played higher after the threat cue (5.93 (2.24)) than after the neutral cue (4.50 (2.53)) on a 0-9 scale, this difference was not significant ($t(29) = 1.72, p = .10$). Furthermore, participants did not report more discomfort when being presented with the threat cue (5.97 (1.77)) rather than with the neutral cue (5.27 (1.82)) on a 0-9 scale, $t(29) = 1.30, p = .21$.

Reaction times

Baseline threat-cuing reaction times were transformed to median reaction times and were subsequently entered into a 2 x 2 (Validity: valid, invalid; Cue type: threat, safe) repeated measures ANOVA. This analysis revealed a main effect of validity, $F(1, 29) = 54.2, p < .00, \eta_p^2 = .65$, showing the basic exogenous cuing effect, such that reaction times were higher for invalid trials ($M = 400.12, SD = 49.22$) compared to valid trials ($M = 364.17, SD = 40.45$). Results are shown in Figure 1. However, there was no significant main effect of cue type, $F < 1$, indicating that reaction times did not differ between neutral cues and threatening cues. Also, no significant interaction effect between validity and cue type, $F < 1$, was found.

Startle response and skin conductance response

Startle magnitude was determined by subtracting the baseline startle responses from the second procedure startle responses. Startle magnitude did not significantly differ between neutral ($M = 23.13, SD = 29.84$) and threatening ($M = 25.76, SD = 33.26$) trials, $t(29) = 1.36, p = .18$. Results are shown in Figure 2. Skin conductance responses were determined by subtracting the baseline SCR responses from the second procedure SCR responses. SCR did not significantly differ either between neutral ($M = 60.85, SD = 126.72$) and threatening trials ($M = 50.70, SD = 78.90$), $t(29) = -.42, p = .67$.

Correlations

The threat-cuing effect did not correlate with the startle effect or the SCR effect, but did show a negative correlation to BIS scores, $r = -.51, p < .005$. When separating the emotional cuing effect into the engagement effect and disengagement effect, only the engagement effect correlated with the startle effect, $r = -.40, p < .05$. This was the only correlation that was found between engagement scores and other measures. Disengagement scores correlated with those on BIS, $r = -.55, p < .005$, BasDrive, $r = .37, p < .05$, and BasFun, $r = .36, p < .05$.

Scores on the STAI were significantly correlated with those on the PSWQ ($r = .60, p < .001$) and the BIS ($r = .47, p < .01$). BIS scores and PSWQ scores were also correlated, $r = .62, p < .001$. Scores on the ACS did not correlate with any other scores, $p < 1$.

Discussion

This study tested several assumptions regarding attentional biases to threat, fear-potentiated startle- and SCR-effects and the association between them. The hypothesis that participants would show a cue validity effect in the emotional cuing task was confirmed. As can be seen in Figure 1, a robust main effect of validity was found, showing that participants were faster to respond to valid cued trials rather than to invalid cued trials. However, all of the other hypotheses were rejected. Because both high and low anxious individuals are assumed to react similar to stimuli of unambiguous threat (Mogg and Bradley, 1998; Mogg et al., 2000), it was expected that all participants would show attentional biases. However, no main effect was found for cue-type, indicating that participants did not differ in the speed of their responses on neutral compared to threatening trials. Furthermore, no interaction effect was found between validity and cue-type, suggesting that the reaction times for neutral and threatening trials did not differ on valid compared to invalid trials. Massar et al. (2010) found that nearly all their participants showed impaired disengagement, regardless of their levels of

trait anxiety. In contrast, participants in the current study did not show impaired attentional disengagement. However, like in the current study, Massar and colleagues did not find increased facilitated engagement effects.

Contrary to Massar and colleagues, who found a clear difference in expectancy, the current study might have failed to create an unambiguous threat situation. A trend was observed, showing that participants thought the aversive sound was more likely to occur after the presentation of the threat cue, rather than the neutral cue, but this trend was not significant. Also, although participants were explicitly informed which cue would be coupled to the aversive sound, they did not differ in their ratings of discomfort of the cues. Possibly, participants were not sure which cue signaled the aversive sound, because the aversive sounds were played both after neutral cues and threatening cues in the preceding startle procedure. Extinction due to the relatively low frequency of reinforcement in the second physiological procedure might also have contributed to the similar ratings of discomfort to the neutral and threatening cue. These methodological issues may explain why no threat-cuing effect was found despite high ratings of aversiveness for the threat cue. Given that participants rated the aversive sound as equally disagreeable as Massars' participants (this study: 7.4 (1.9); Massars study: 7.5(1.5)), it seems improbable that the aversive sound was not strong enough to induce threat in the participants.

The hypothesis that participants' threat cuing effect would be positively associated to the magnitude of their startle- and SCR-effect could not be confirmed either. Neither the threat-cuing effect nor the SCR effect showed any relation with the other measures. The latter finding is not entirely surprising, because SCR can be a fairly unspecific measure with a high level of noise. When separating the emotional cuing effect into threat effects on valid trials (engagement) and invalid trials (disengagement), only the engagement effect was related to the startle effect. However, contrary to expectations, this was a negative relationship. This

finding indicates that participants that reacted faster to threatening cues showed weaker fear potentiated startle effects. These findings contrast with the expectations, but might be explained by a methodological shortcoming. Because of the large amount of pairwise comparisons made, this significant negative correlation might have been the result of a cumulative type I error, indicating a significant correlation where there isn't one.

Furthermore, the prediction that participants' level of trait anxiety would be positively linked to the engagement effect, and to the magnitude of their startle- and skin conductance response could not be confirmed. Participants did not show any association between their levels of trait anxiety and (dis)engagement effects. Moreover, trait anxiety scores were not associated with the startle- and SCR-effect. The distribution of trait anxiety scores was positively skewed (skewness = 1.25), indicating that few high anxious individuals participated in the study. On a group level, the sample of men scored just below average for men (44th percentile) and the sample of women just above average for women (53rd percentile) relative to the STAI trait anxiety norms for college students (Spielberger et al., 1970). It is possible that despite explicit affirmation that no electric shocks would be administered in the current experiment, the anticipation of receiving shocks further on in the experiment might have put people more on edge than they would normally have been. Compared to the anticipated shock, the aversive noise might have had a relatively smaller impact.

The next expectation was to find associations between self-reported levels of BIS and BAS, attentional biases and the startle- and SCR-effect. The unexpected finding was that BIS scores were negatively related to the disengagement effect. This is surprising, because the positive relationship between disengagement and anxiety is fairly robust. Numerous studies using the spatial cuing task have shown that anxious individuals have difficulty in disengaging from threat cues (e.g., Cisler & Olatunji, 2010; Koster, Crombez, Verschuere, Van Damme, et al., 2006; Van Damme, Crombez, Hermans, Koster, & Eccleston, 2006;

Wilson & MacLeod, 2003). However, these studies defined anxious individuals as having high trait anxiety scores rather than having high BIS scores. Although BIS scores are related to trait anxiety scores, BIS scores reflect an individual's predisposition to anxiety rather than the experience of anxiety (Jorm et al., 1998). Using the same paradigm as the one employed in the current study, Massar and colleagues (2010) found that BIS scores and disengagement scores were positively associated. They propose that because BIS causes an individual to stop current actions when encountering a threat, the relative slowing of responses to invalidly cued targets may be caused by the inhibition of task-relevant attention. However, in the current study, participants did not show any inhibition of task-relevant attention. Rather, participants with more behavioral inhibition had fewer problems directing their attention away from the invalidly placed threatening cue. The BasDrive showed a significant positive correlation with the threat cuing effect. As expected, trait anxiety and worry were positively associated. However, the attentional control scale did not correlate with trait anxiety or any of the attentional measures.

Despite careful replication of the study by Massar et al. (2005), further methodological shortcomings of the present study can be mentioned. Firstly, the way participants were recruited might have influenced the composition of the sample. The flyers that were used to motivate students to participate in the study mentioned that participants would be receiving light electric shocks. These shocks were not administered in the current study, but in a procedure that was linked to, and immediately followed it. Possibly, this has prevented individuals high in trait anxiety from participating. Also, due to several methodological issues, the final sample consisted of merely 30 participants, compared to 57 in the original study by Massar and colleagues (2010). A larger sample would have been more representative of the general population. Another methodological problem might have been that the baseline emotional cuing task was followed by the physiological baseline procedure before

moving on to the second emotional cuing task. Though the baseline cuing task did not include aversive noises, the baseline physiological procedure did. Possibly, the baseline physiological procedure might have put participants more on edge than would have been the case if the baseline cuing task was directly followed by the second cuing task. Another possibility is that participants were not able to distinguish between neutral and threatening cues because the noises were also played when neutral cues were presented in the physiological procedure that proceeded the second cuing task.

Research on attentional biases to threat has made substantial progression during the last decade. We have gained important insight in the components that make up attentional biases and the influence that threat anxiety exerts on them. Although attentional biases occur in all anxiety disorders, they are not specific to any disorder, but represent a component of high trait anxiety (Cisler & Koster, 2010). The creation of distinct groups of HTA and LTA individuals might yield stronger results regarding the relationship between attentional biases, fear potentiated startle and trait anxiety. Much is still to discover about the association between attentional biases and physiological expressions of anxiety. This study has not been able to provide straight-forward answers to the posed questions. The association between amygdalar functioning and attentional biases is still largely unknown and warrants further investigation. Because in the current study both startle- and skin conductance responses were measured, the experimental procedure might have had limited power to measure either response. Prospective studies could measure these responses in distinct studies. This would allow for the presentation of more startle trials, which might yield stronger results.

References

- Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, & van IJzendoorn, M. H. (2007). Threat-related attentional bias in anxious and non-anxious individuals: A meta-analytic study. *Psychological Bulletin*, *133*, 1-24. doi: 10.1037/0033-2909.133.1.1
- Bishop, S. J. (2008). Trait anxiety and impoverished prefrontal control of attention. *Nature Neuroscience*, *12*(1), 92- 98. doi:10.1038/nn.2242
- Büchel, C., Morris, J., Dolan, R.J., & Friston, K. J. (1998). Brain systems mediating aversive conditioning, an event-related fMRI study. *Neuron*, *20*, 947–957. doi:10.1016/S0896-6273(00)80476-6
- Campeau, S., Davis, M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *Journal of Neuroscience*, *15*, 2301–2311.
- Carver, S. C., & White, T. L. (1984). Behavioral activation, and affective responses to impending reward and punishment: the bis/bas scales. *Journal of Personality and Social Psychology*, *67*, 319-333. doi: 10.1037/0022-3514.67.2.319
- Cheng, D.T., Knight, D.C., Smith, C. N., Stein, E.A., & Helmstetter, F.J. (2003). Functional MRI of human amygdala activity during Pavlovian fear conditioning: stimulus processing versus response expression. *Behavioural Neuroscience*, *117*, 3-10. doi: 10.1037/0735-7044.117.1.3
- Cisler, J. M., & Koster, E. H. W. (2010). Mechanisms of attentional biases towards threat in anxiety disorders: An integrative review. *Clinical Psychology Review*, *30*, 203-216. doi: 10.1016/j.cpr.2009.11.003
- Cisler, J. M., & Olatunji, B. O. (2010). Components of attentional biases in contamination fear: Evidence for difficulty in disengagement. *Behaviour Research and Therapy*, *48*, 74-

78. doi:10.1016/j.brat.2009.09.003

Constans, J. I. (2001). Worry propensity and the perception of risk. *Behaviour Research and Therapy*, *39*, 721-729. doi:10.1016/S0005-7967(00)00037-1

Critchley, H.D. , Mathias, C.J., & Dolan, R. J. (2002). Fear conditioning in humans, the influence of awareness and autonomic arousal on functional neuroanatomy. *Neuron*, *33*, 653–663. doi:10.1016/S0896-6273(02)00588-3

Davey, G. C. L. (1994). Worrying, social problem-solving abilities, and social problem-solving confidence. *Behaviour Research and Therapy*, *32*, 327-330. doi:10.1016/0005-7967(94)90130-9

Davis, M. (1986). Pharmacological and anatomical analysis of fear conditioning using the fear-potentiated startle paradigm. *Behavioral Neuroscience*, *100*, 814–824. doi:10.1037/0735-7044.100.6.814

Derryberry, D., & Reed, M. A. (2002). Anxiety-Related Attentional Biases and Their Regulation by Attentional Control. *Journal of Abnormal Psychology*, *111*(2), 225. doi: 10.1037//0021-843X.111.2.225

Eysenck, M. W. (1997). *Anxiety and cognition: a unified theory*. Psychology Press: Hove.

Eysenck, M. W. (2000). A cognitive approach to trait anxiety. *European Journal of Personality*, *14*, 463- 476. doi: 10.1002/1099-0984(200009

Fischer, H., Andersson, J.L., Furmark, T., Wik, G., & Fredrikson, M. (2002). Right-sided human prefrontal brain activation during acquisition of conditioned fear. *Emotion*, *2*, 233–241. doi: 10.1037/1528-3542.2.3.233

Fox, E., Russo, R., & Dutton, K. (2002). Attentional bias for threat: Evidence for delayed disengagement from emotional faces. *Cognition & Emotion*, *16*, 355- 379. doi: 10.1080/02699930143000527

Gray JA, McNaughton N. (2000). *The Neuropsychology of anxiety: an enquiry into the*

functions of the septo-hippocampal system, 2nd ed. Oxford: Oxford University Press.

Grillon, C., Ameli, R., Woods, S. W., Merikangas, K., & Davis, M. (1991). Fear potentiated startle in humans: Effects of anticipatory anxiety on the acoustic blink reflex.

Psychophysiology, 28, 588-595. doi: 10.1111/j.1469-8986.1991.tb01999.x

Grillon, C., & Baas, J. M. (2003). A review of the modulation of startle by affective states and its application to psychiatry. *Clinical Neurophysiology*, 114, 1557–1579.

doi:10.1016/S1388-2457(03)00202-5

Heubeck, B. G., Wilkinson, R. B., & Cologon, J. (1998). A second look at Carver and White's (1994) BIS/BAS scales. *Personality and Individual Differences*, 25, 785-800.

doi:10.1016/S0191-8869(98)00124-X

Hitchcock, J.M., Davis, M. (1986). Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm.

Behavioral Neuroscience, 100, 11–22. doi:10.1037/0735-7044.100.1.11

Hitchcock, J.M., Davis, M. (1987). Fear-potentiated startle using an auditory conditioned stimulus: effect of lesions of the amygdala. *Physiological & Behavior*, 39, 403–408.

doi:10.1016/0031-9384(87)90242-3

Jorm, A. F., Christensen, H., Henderson, A. S., Jacomb, P. A., Korten, A. E., & Rodgers, B.

(1998). Using the BIS/BAS scales to measure behavioural inhibition and behavioural activation: Factor structure, validity and norms in a large community sample. *Personality and Individual Differences*, 26(1), 49-58. doi:10.1016/S0191-8869(98)00143-3

doi:10.1016/S0191-8869(98)00143-3

Knight, D.C., Smith, C.N., Stein, E.A., & Helmstetter, F. J. (1999). Functional MRI of human Pavlovian fear conditioning, patterns of activation as a function of learning.

NeuroReport, 10, 3665–3670. doi: 10.1097/00001756-199911260-00037

Knight, D.C., Cheng, D.T., Smith, C.N., Stein, E.A., & Helmstetter, F. J. (2004). Neural

substrates mediating human delay and trace fear conditioning. *Journal of Neuroscience*,

24, 218–228. doi:10.1523/JNEUROSCI.0433-03.2004

Koster, E. H., Crombez, G., Van Damme, S., G., Verschuere, & De Houwer, J. (2004). Does imminent threat capture and hold attention? *Emotion*, *4*(3), 312-317. doi: 10.1037/1528-3542.4.3.312

Koster, E. H., Crombez, G., Verschuere, B., Van Damme, S., & Wiersema, J. R. (2006). Components of attentional bias to threat in high trait anxiety: Facilitated engagement, impaired disengagement, and attentional avoidance. *Behaviour Research and Therapy*, *44*(12), 1757-1771. doi:10.1016/j.brat.2005.12.011

Lang, P. J., Davis, M., & Öhman, A. (2000). Fear and anxiety: animal models and human cognitive psychophysiology. *Journal of Affective Disorders*, *61*, 137 – 159.
doi: 10.1016/S0165-0327(00)00343-8

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1990). Emotion, attention, and the startle reflex. *Psychological Review*, *97*(3), 377-395. doi: 10.1037/0033-295X.97.3.377

Li, L., Steidl, S., Yeomans, J.S. (2001). Contributions of the vestibular nucleus and vestibulospinal tract to the startle reflex. *Neuroscience* *106*, 811–821. doi:10.1016/S0306-4522(01)00324-4

Li, L., Yeomans, J.S. (1999). Summation between acoustic and trigeminal stimuli evoking startle. *Neuroscience* *90*, 139–152. doi:10.1016/S0306-4522(98)00436-9

MacLeod, C., Mathews, A., & Tata, P. (1986). Attentional bias in emotional disorders. *Journal of Abnormal Psychology*, *95*, 15-20. doi:10.1037/0021-843X.95.1.15

Massar, S. A. A., Mol, N. M., Kenemans, J. L., & Baas, J. M. P. (2010). Attentional bias in high- and low- anxious individuals: Evidence for threat-induced effects on engagement and disengagement. *Cognition & Emotion*, First published on 13 october 2010.
doi:10.1080/02699931.2010.515065

Meyer, T. J., Miller, M. L., Metzger, R. L., & Borkovec, T. D. (1990). Development and

- validation of the Penn State Worry Questionnaire. *Behaviour Research and Therapy*, 28, 487–495. doi:10.1016/0005-7967(90)90135-6
- Mogg, K., & Bradley, B. P. (1998). A cognitive motivational analysis of anxiety. *Behaviour Research and Therapy*, 36, 809- 848. doi:10.1016/S0005-7967(98)00063-1
- Mogg, K., McNamara, J., Powys, M., Rawlinson, H., Seiffer, A., & Bradley, B. P. (2000). Selective attention to threat: A test of two cognitive models of anxiety. *Cognition & Emotion*, 14, 375-399. doi: 10.1080/026999300378888
- Morris, J.S , Buchel, C., & Dolan, R. J. (2001). Parallel neural responses in amygdala subregions and sensory cortex during implicit fear conditioning, *NeuroImage* , 13, 1044–1052. doi:10.1006/nimg.2000.0721
- Öhman, A. (2005). The role of the amygdala in human fear: Automatic detection of threat. *Psychoneuroendocrinology*, 30, 953- 958. doi:10.1016/j.psyneuen.2005.03.019
- Öhman, A., Flykt, A., & Lundqvist, D. (2000). Unconscious emotion: Evolutionary perspectives , psychophysiological data, and neuropsychological mechanisms. In R.D. Lane & L. Nadel (Eds.), *Cognitive neuroscience of emotion* (pp. 296-327). New York: Oxford University Press.
- Peers, P. V., & Lawrence, A. D. (2009). Attentional control of emotional distraction in rapid serial visual presentation. *Emotion*, 9, 140- 145. doi:10.1037/a0014507
- Phelps, E.A. , Delgado, M.R., Nearing, K.I., & LeDoux, J. E. (2004). Extinction learning in humans, role of the amygdala and vmPFC. *Neuron*, 43, 897–905. doi:10.1016/j.neuron.2004.08.042
- Posner, M. I., Inhoff, A., Friedrich, F. J., & Cohen, A. (1987). Isolating attentional systems: A cognitive-anatomical analysis. *Psychobiology*, 15, 107- 121.
- Reinholdt-Dunne, M. L., Mogg, K., & Bradley, B. P. (2009) Effects of anxiety and attention control on processing pictorial and linguistic emotional information. *Behaviour*

- Research and Therapy*, 47 (5), 410-417. doi:10.1016/j.brat.2009.01.012
- Spielberger, C. D., Gorsuch, R., & Lushene, R. (1970). *The State-Trait Anxiety Inventory (STAI) Test Manual Form X*. Consulting Psychologists Press: Palo Alto, Ca.
- Tremayne, P., & Barry, R. J. (2001). Elite pistol shooters: physiological patterning of best vs. worst shots. *International Journal of Psychophysiology* 4, 19-29. doi:10.1016/S0167-8760(00)00175-6
- Van Damme, S., Crombez, G., Hermans, D., Koster, E. H. W., & Eccleston, C. (2006). The role of extinction and reinstatement in attentional bias to threat: A conditioning approach. *Behaviour Research and Therapy*, 44, 1555- 1563. doi:10.1016/j.brat.2005.11.008
- van der Ploeg, H. M., Defares, P. B., & Spielberger, C. D. (1980). Een Nederlandstalige bewerking van de Spielberger state-trait anxiety inventory STAI-DY. *De psycholoog*, XV, 8, 460-467.
- Vrana, S. R., Spence, E. L., & Lang, P. J. (1988). The startle probe response: A new measure of emotion? *Journal of Abnormal Psychology*, 97, 487– 491. doi: 10.1037/0021-843X.97.4.487
- Waters, A.M., Lipp, O.V., Spence, S. (2005). The effects of affective picture stimuli on blink modulation in adults and children. *Biological Psychology*, 68, 257–281. doi:10.1016/j.biopsycho.2004.05.002
- Wells, A. (1994). A multi-dimensional measure of worry: Development and preliminary validation of the Anxious Thoughts Inventory. *Anxiety, Stress, and Coping*, 6, 289-299. doi: 10.1080/10615809408248803
- Williams, J. M. G., Mathews, A., & MacLeod, C. (1996). The emotional Stroop task and psychopathology. *Psychological Bulletin*, 120, 3- 24. doi: 10.1037/0033-2909.120.1.3
- Wilson, E., & MacLeod, C. (2003). Contrasting two accounts of anxiety-linked attentional bias: Selective attention to varying levels of stimulus threat intensity. *Journal of Abnormal*

Psychology, 112, 212- 218. doi: 10.1037/0021-843X.112.2.212

Yiend, J., & Mathews, A. (2001). Anxiety and attention to threatening pictures. *Quarterly Journal of Experimental Psychology*, 54A, 665-681. doi: 10.1080/02724980042000462

Figure 1. Reaction time data. Bars and error bars represent mean RT and standard error of the mean.

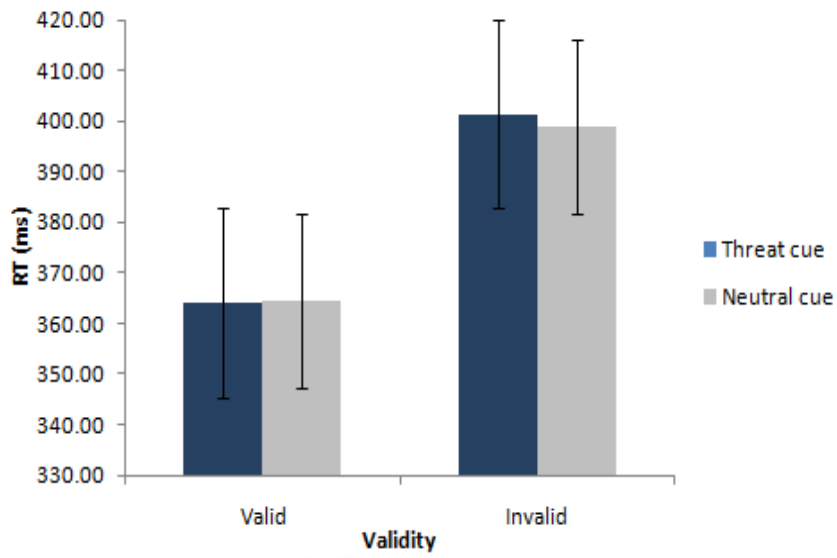


Figure 2. Fear potentiated startle data. Bars and error bars represent startle magnitude and standard error of the mean.

