Bachelor Thesis

Investigating Avoidance Behavior and Its Effects on Fear-Potentiated Startle Using a Novel Avoidance Paradigm

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Abstract

Previous research on avoidance in fear conditioning has focused on relatively simple tasks that require little action (e.g. a single button press). Little is known about the effects of task difficulty and the level of vigor necessary to complete avoidance on the physiologically conditioned fear response. In the present pilot study, we used a novel avoidance paradigm to investigate how varying the distance to a safety area affects avoidance behavior and fear-potentiated startle (FPS). After conditional fear acquisition with a shock as the unconditioned stimulus (US), the participants were able to avoid the shock by maneuvering a figure into the safety area during a confined movement period. Startle amplitude was measured both before and after the movement period. We hypothesized that the effects of distance to safety would be comparable to those of proximity to threat. The comparison proved to be highly difficult as the startle reflex appeared to be affected by a variety of factors that could not be controlled for. Individual variability with regard to movement behavior was found. We conclude that the current avoidance paradigm appears to be unsuitable for examining FPS; nonetheless, it could be useful in investigating individual and group differences in avoidance behavior.

Introduction

Fear conditioning is a paradigm in which a previously neutral stimulus is paired with an unconditioned aversive stimulus (US) (e.g. an electrical shock). By coupling the two, the neutral stimulus becomes conditioned (CS) and the participant is able to predict the aversive stimulus. The CS induces fear due to the link between the two stimuli. Multiple studies have investigated the mechanisms related to avoiding the aversive stimulus (Delgado et al., 2009; Dymond et al., 2012). In a typical active avoidance paradigm, the participant is able to eliminate the US via a specific action, for example by pressing a certain button at the right moment.

To our knowledge, nobody has yet examined how the level of vigor of the action necessary for successful avoidance affects physiological conditioned fear responses. The level of vigor could be simulated by varying the distance to a safety area, which the participant would need to reach in a restricted amount of time. This could be comparable to proximity to threat. McNaughton & Corr (2004) developed a neuropsychological model of the neural systems controlling defense that incorporates distance to threat. The model has two different dimensions, namely defensive direction and defensive distance. The defensive direction can be either avoidance, when leaving a threat or dangerous situation, or approach, when entering such a situation. In the context of this study, only one direction, defensive avoidance, is of relevance. Defensive distance is not real distance per se, but describes the subjective intensity of the perceived threat; thus, a subjectively highly threatening stimulus may be considered more proximal in terms of defensive distance than a less threatening stimulus that is closer in real distance. Defensive distance controls the type of behavior that occurs and, therefore, also its neural underpinnings. McNaughton & Corr (2004) divide the behavior as well as the respective brain regions into hierarchical levels according to defensive distance. At intermediate distances, the amygdala is involved in active avoidance and arousal, at shorter distances, the medial hypothalamus controls directed escape, and at highly close proximity, undirected escape/panic is mediated by the periaqueductal gray. Generally speaking, the amygdala-mediated fear response is activated as a result of stimuli or situations perceived as dangerous; however, threatening stimuli that are more proximal in terms of defensive distance appear to induce a fight-or-flight response that involves other neural mechanisms (McNaughton & Corr, 2004). In other words, when defensive distance to threat is intermediate, the amygdala mediates the fear response as well as active avoidance; short defensive distance to threat requires more rapid action and, therefore, results in the activation of other brain regions.

This is in line with studies that investigated the effects of proximity to threat in humans. Using looming picture sequences, Löw et al. (2008) found a reduction in the fear-potentiated startle response (FPS), a measurement closely related to amygdala activity, and an increase in skin conductance response (SCR) and heart rate (HR) with increasing proximity of a threat (money loss). The results have to be regarded with caution as Delgado et al. (2011) showed that the amygdala is active during fear conditioning with primary reinforcers (mild shock), but not during conditioning with secondary reinforcers (loss of money). FPS is mediated by the amygdala (e.g. Pissiota et al., 2003); thus, the decrease in FPS with closer proximity found by Löw et al. (2008) may be due to less amygdala activity during loss of money. Nonetheless, there are other studies suggesting that distinct neural systems are activated in response to distal and proximal threats. Using a virtual predator, Mobbs et al. (2007) discovered that high threat distance induces activity in the vmPFC, whereas high threat proximity elicits activity in the PAG, a region implicated in fight and flight. Although no direct activation of the amygdala was found, the authors argue that vmPFC activity during distant threats could be indicative of involvement of the amygdala due to neural connections between the two areas. Mobbs et al. (2009) obtained similar results for potential and imminent danger. Early anticipation of an aversive event led to increased activity in forebrain structures including the amygdala and imminent threat induced activity in midbrain regions known to be involved in panic and analgesia, e.g. the PAG.

The effects of close proximity to threat or imminent danger may be comparable to those of having a large distance to safety. In both cases, it is crucial to react quickly to a threatening situation. Due to the fact that avoidance of the aversive US is difficult, large distance to safety requires rapid and quick avoidance behavior and high level of vigor similar to fight or flight. Therefore, it may induce activity in the PAG. When safety is close, the speed of movement is not as essential and the level of vigor needed to avoid the US is much smaller. Thus, it may result in the activation of a different neural system that includes the amygdala.

Evidence suggests that the acoustic startle reflex is a good indicator for amygdala activation and acquired fear from aversive conditioning (Davis, 1992; Hamm et al., 1993; Lipp et al., 1994). Fear-potentiated startle (FPS) is mediated by the amygdala in both rodents (Hitchcock & Davis, 1986) and humans (Pissiota et al., 2003) and therefore has high construct validity as a measurement of fear (Norrholm et al., 2006). Nonetheless, previous literature suggests that the startle reflex is inhibited when a reaction time cue is anticipated suggesting that less attention is diverted to the task-irrelevant startle stimulus (Anthony, 1985; Löw et al., 2008).

The current pilot study investigated how distance to safety and avoidance behavior towards safety affect FPS using a novel avoidance fear conditioning paradigm. The experiment consisted of an Acquisition and an Avoidance phase. During Acquisition, one conditioned stimulus was paired with an electric shock (CS+) while the other one was not (CS-); during Avoidance, participants were able to avoid the shock by moving a figure, which had different starting locations, into a safety area on the right side of the screen when indicated by a cue. FPS was measured both before the movement started (early startle) as well as after the movement had finished (late startle) and movement behavior in the form of button presses was recorded.

Based on previous literature on distance to threat, we expected high potentiation of early startle when the starting position of the figure was in close proximity to the safety area and a relative inhibition of early startle when the figure started further away from the safety area. When the distance between figure and safety was highest, it would be highly difficult to avoid the shock; therefore, we predicted startle potentiation to increase in this condition as a result of higher shock expectancy. Secondly, as late startle was measured after the participants stopped moving, we predicted that startle would be higher if they did not move into the safety area, which would be most probable when the starting position was far away from safety. Lastly, we expected startle amplitudes in CS- trials to not differ between conditions and to be lower than during CS+ trials due to the fact that the CS- was not paired to the shock.

Methods

Participants

10 subjects participated in the study (3 female, 7 male). Each participant gave written consent prior to the experiment and received 8 euros after completion. The Ethical Committee of the Faculty of Social Sciences of Utrecht University approved all procedures used in the experiment.

Stimuli

At the beginning of each trial, a figure was displayed in front of a background (see figure 1). Two different background colors served as the CSs (CS-: green, CS-: blue; see fig. 1). The right quarter of the background remained grey throughout the whole experiment as it served as the safety area. The figure represented the participant.

The US was an electric shock consisting of a 625 ms train of 5 ms pulses. The shock was produced via a constant current simulator and delivered through an electrode on the participant's left wrist. To individually set the shock level, each participant underwent a work-up procedure. The level was adjusted according to participant's ratings of nine sample shocks to achieve an intensity that was rated as "quite annoying". Startle probes were 110 dB white noise bursts lasting 50 ms presented through headphones.

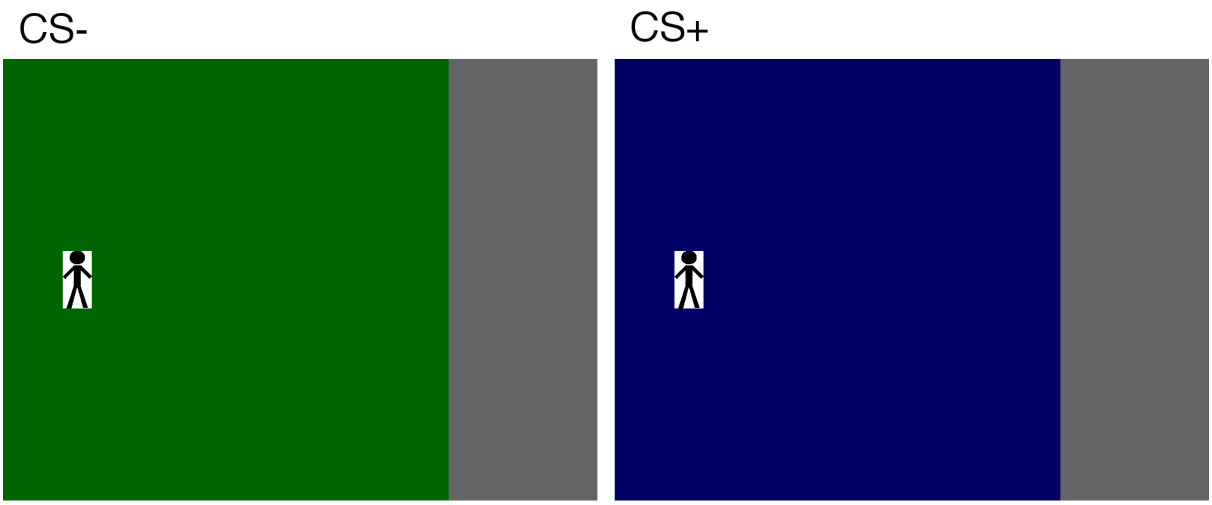

Fig. 1. Screenshots of the beginning of a CS- and the beginning of a CS+ trial. The green background served as the CS- (left) and the blue background as CS+ (right). The right grey quarter of the screen constituted the safety area. The stick figure on the left of both screenshots started in one of four positions at the beginning of each trial (see Fig. 2) and could be moved during Avoidance.

Measurements

The Biosemi Active Two system was used to record and amplify fear-potentiated startle (FPS). The eye-blink reflex as a response to the startle probe was measured using two electrodes placed under the right eye ±15mm apart. All electrodes were filled with standard electrolyte gel. Startle was either not measured at all (no startle condition), 2.5s post-trial-onset (early startle) or 8.5s post-trial-onset (late startle).

The Inter Startle Interval (ISI) was always equal to or larger than 18s and there were always at least 10 seconds between the last shock and the following startle measurement. The Inter Trial Interval (ITI) was adjusted according to these confinements.

Procedure

The experiment consisted of two distinct phases, namely Acquisition and Avoidance. Before the Acquisition phase, participants were first informed about the relationship between CS+ and the shock. Thereafter, a series of 12 trials were presented in a pseudo-random order. During Acquisition, the first three out of four figure positions were used (Fig. 2). See below for more detail on these positions. The conditioned stimulus (either CS+ or CS-) was presented simultaneously to the start of the trial. Each trial lasted 10 seconds. A 625ms shock followed CS+ trials, while CS- trials were followed by a 625ms break. The reinforcement rate was 100%. The duration of the ITI was as described in the measurements section. In between trials, the sentence "Get ready for the next trial!" was displayed on the screen.

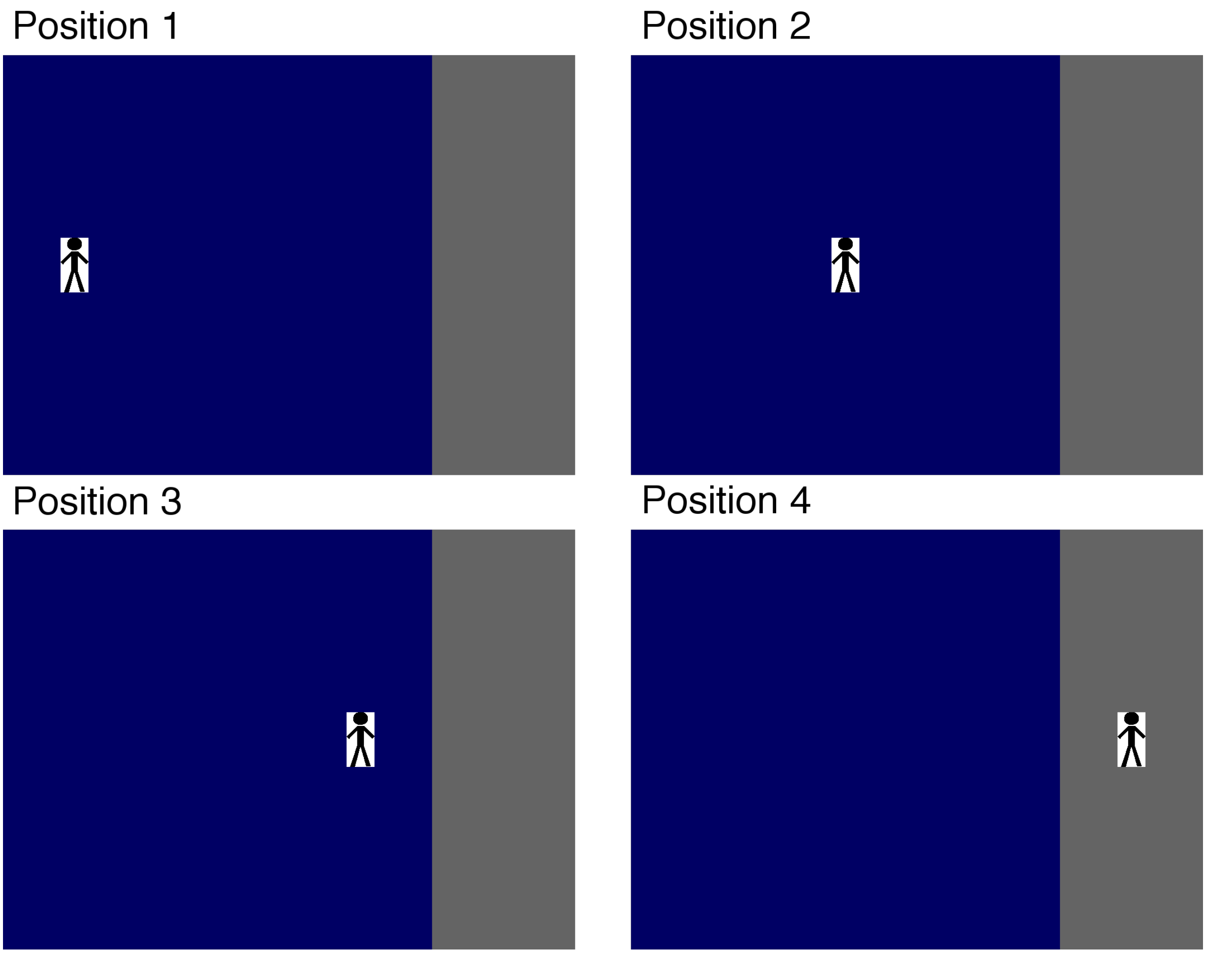

Fig. 2. Starting positions of the figure. At the beginning of each trial, the figure could be located in one of four different places on the screen (position 1, position 2, position 3 & position 4). Position 1 represented the center of the far left quarter of the screen, position 2 the center of the central left quarter, position 3 the center of central right quarter and position 4 the center of the far right quarter. During Acquisition only starting positions 1 to 3 were used.

	Early Startle		Late Startle	
	CS+	**CS-**	**CS+**	**CS-**
Position 1	9	9	3	3
Position 2	9	9	3	3
Position 3	9	9	3	3
Position 4	9	9	3	3

Table 1. Different conditions during Avoidance phase. During Avoidance, there were eight different conditions made up of four positions (1-4) and two conditioned stimuli (CS- or CS+). Participants did a total of 128 trials. 16 trials per condition were separated into 9 early startle, 3 late startle and 4 no startle trials as seen above.

Before the start of Avoidance, the participants were instructed that they could avoid the shock during CS+ trials by moving the figure into the grey safety area on the right when indicated by a picture cue. First, the participants did four practice trials that included all four positions, both CS+ and CS-, and all different startle conditions. Thereafter, a total of 128 trials (32 no startle, 72 early startle, 24 late startle) were presented in a pseudo-random order. Thus, there were 9 early startle and 3 late startle measurements per condition. No startle trials made up 1/4 of the total number of trials. As during Acquisition, each trial had a duration of 10 seconds. The startle measurements were taken as described in the measurements section. Each trial started with a presentation of the CS+ or CS- and the figure in one of four positions: middle of the far left quarter of the screen (position 1), middle of the central left quarter of the screen (position 2), middle of the central right quarter of the screen (position 3) or middle of the far right quarter of the screen and, thus, already inside the safety area (position 4) (Fig. 2). This resulted in a total of eight different conditions consisting of a combination of the two conditioned stimuli and the four different starting locations (Table 2).

A picture cue (see Fig. 3) located in the top center of the screen signaled the start of the movement period during which the participant could use the arrow keys to maneuver the figure into the safety area. The cue stayed on screen during the entire length of the movement period. The duration was consistent across trials (3s), but the onset of the movement period was jittered and varied between conditions. This made the onset unpredictable which means that subjects started to anticipate the moving period from the beginning of the trial, while also making startle measurements during different phases of the trial possible (no startle: movement onset between 0.5s and 4.5s; early startle: between 3.5s and 4.5s; late startle: between 0.5s and 1.5s; see Fig. 3). During CS+ trials, no shock was delivered if the whole figure was inside the safety area at the end of the movement period; otherwise, a 625ms shock followed the end of the trial. CS- presentation was never coupled to a shock. The structure of the different types of trials including the timing is illustrated in Figure 3. The speed of movement was 25 pixels per button press (screen size: 1024x768) such that it was highly difficult to avoid the shock starting from position 1, moderately difficult from position 2 and easy from position 3. Position 4 did not require any movement to avoid the shock. The amount and the direction of button key presses as well as the success or failure of avoiding the US were recorded.

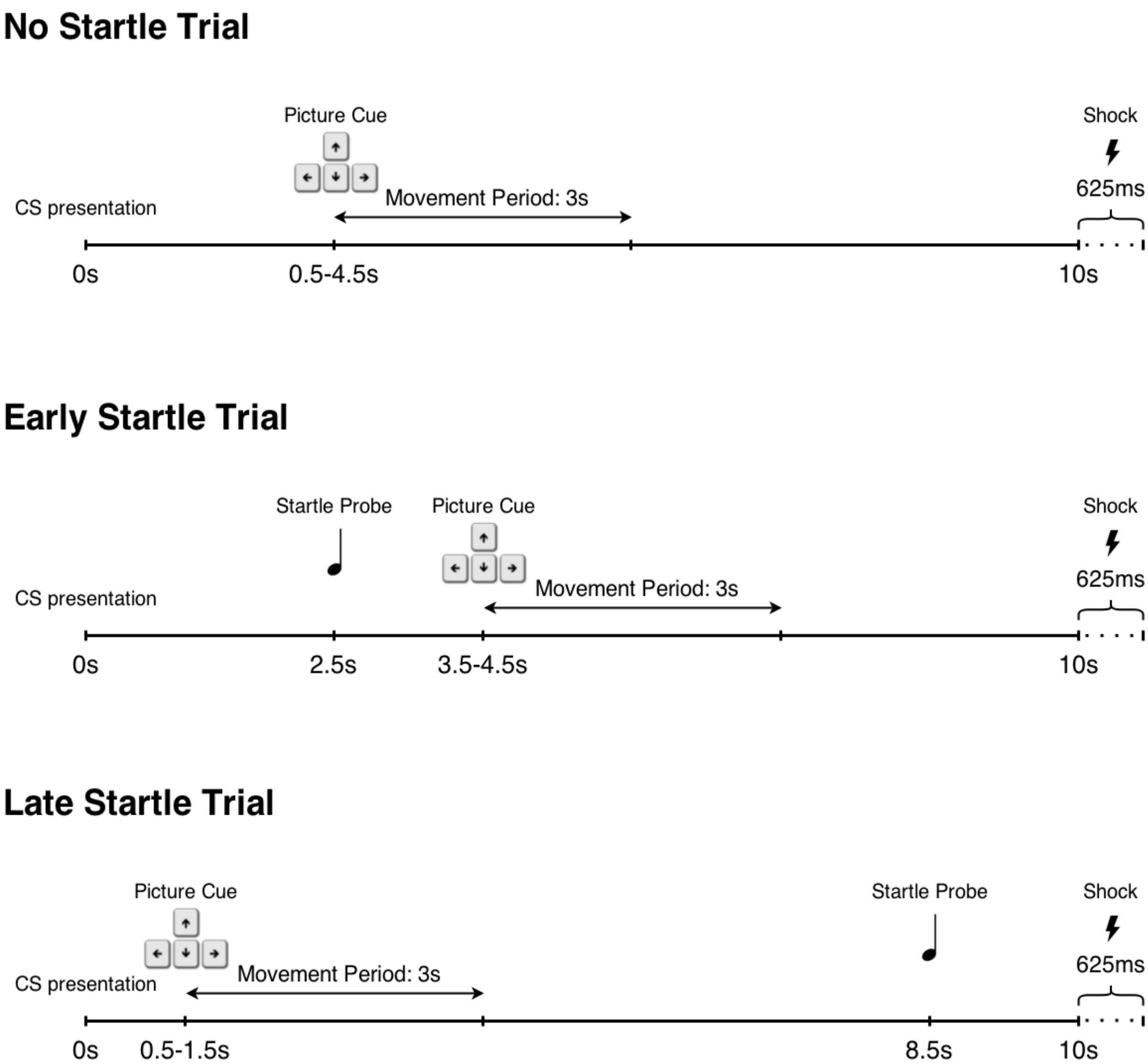

Fig. 3. Timing of no startle, early startle and late startle trials. Each of the three types of trials started with the CS presentation and included a movement period of 3 seconds. Arrow keys were displayed throughout the duration of the movement period and served as a picture cue. The onset time of the movement period was jittered and differed for the three types of trials (no startle: 0.5-4.5s; early startle: 3.5-4.5s; late startle 0.5-1.5s). During early startle trials, the startle probe occurred before the movement period (at 2.5s) and during late startle trials, the startle probe occurred after the movement period (at 8.5s). At the end of CS+ trials, participants received an electric shock if they did not manage to move the figure into the safety area on time.

Data Analysis

To compare the different conditions with regard to movement behaviour, the amount of button presses per condition was averaged per participant and, subsequently, the average and standard deviation was calculated for each condition. In addition, the difficulty of the different CS+ condition was evaluated by examining how many times the condition was avoided successfully.

The physiological data were processed using Vision Analyzer software. The data were segmented into epochs starting from 50ms before startle probe onset and ending 200ms post-onset. A 28 Hz low pass filter and a 500 Hz high pass filter were applied. Subsequently, the segments were rectified, smoothed using a 14 Hz high pass filter and baseline-corrected. Startle magnitude was defined as the highest peak 25-140ms post-onset. Thereafter, all segments were manually checked for artefacts: all trials with a baseline activity substantially higher than the mean baseline of the participant were rejected. Startle data were z-transformed based on all measurements per participant and the resulting z-scores were used for all analyses.

SPSS Version 22 for Windows was used for statistical analysis. First, a repeated measures ANOVA with the factors startle time (early or late), position (1-4), and CS (+ or -) and the dependent variable startle amplitude was performed. Subsequently, early and late startle measurements were analyzed separately; the same procedure was followed for both. Two repeated measures ANOVAs with the factors position and CS were conducted. When a significant effect was found, follow-up analyses were applied to further characterize the effect.

Results

One of 10 participants was excluded from the analysis as a result of too little available data due to a measurement error.

Avoidance Behavior

The difficulty of avoiding the US during CS+ trials from each of the four positions differed from person to person. Six of the nine participants included in the analysis were not able to avoid the shock in position 1 trials whereas the other three could sometimes avoid the shock. Two of the three even managed to avoid in more than half of position 1 trials, one of which used two fingers in position 1 trials to be fast enough to avoid the shock and informed the experimenter after the study was over. Four of the nine participants always avoided the shock in position 2 trials; the other five failed at least once. Almost all participants avoided all shocks in position 3 and position 4 trials; one participant did not avoid a single position 3 trial and another didn't avoid a total of four position 3 trials and even left the safety area in two position 4 trials. Although this represents a deviation from the expected avoidance behavior, data from the participant in question was included in the analysis due to the fact that this deviation did not occur in the majority of trials (75% in position 3 and 87.5% in position 4).

The average number of button presses and its standard deviation for each condition are displayed in Table 2. The values include all movement directions. The minimum amount of right arrow key presses needed to avoid a shock in CS+ trials was 27 in position 1, 17 in position 2, and 7 in position 3. Average amount of button presses appears higher for CS+ conditions, whereas standard deviation appears to be higher for CS- conditions. This shows that there were large individual differences with regard to movement during CS- trials: three subjects showed close to no movement (less than 4% of trials) whereas the other subjects moved similarly in CS+ and CS- conditions or showed no specific movement patterns.

	CS- position 1	**CS- position 2**	**CS- position 3**	**CS- position 4**
Mean	8.1	9.6	6.5	4.5
Standard Deviation	7.5	7.7	5.5	5.3
	CS+ position 1	**CS+ position 2**	**CS+ position 3**	**CS+ position 4**
Mean	19.8	17.9	9	3.9
Standard Deviation	5.3	2.3	2.2	4.4

Table 1. Average amount and standard deviation of button presses per condition. Values include all four possible arrow key presses.

Fear-Potentiated Startle

A repeated measures analysis with the factors startle time (early & late), position (1-4), and CS (+ & -), resulted in significant main effects of startle time ($F(1,5)=18.474$, $p=.008$) and position ($F(3,15)=12.631$, $p<.001$), while the main effect of CS approached significance ($F(1,5)=4.948$, $p=.077$). In addition, interaction effects between the three factors were significant (startle time x position, $F(3,15)=10.938$, $p<.001$; startle time x CS, $F(1,5)=8.945$, $p=.03$; position x CS, $F(3,15)=5.586$, $p=.009$). The results are shown in Fig. 4.

To investigate early and late trials separately, two repeated measures analyses with the factors position and CS were conducted. Whereas no significant main or interaction effects were found in the early startle analysis (position: Greenhouse Geisser correction due to violation of assumption of sphericity ($\varepsilon=.53$), $F(1.316, 10.529)=.567$, $p=.642$; CS, $F(1,8)=.002$, $p=.969$), late startle analysis revealed significant main effects for position ($F(3,15)=16.852$, $p<.001$) and CS ($F(1,5)=7.089$, $p=.045$) and a significant interaction effect between position and CS ($F(3,15)=4.379$, $p=.021$).

To further characterize the nature of the startle effects measured after the movement period (late startle), two repeated measures ANOVAs using position as a factor and either CS- late startle measurements or CS+ late startle measurements as the dependent variable were conducted. Both analyses showed a significant main effect of position (CS-: $F(3,21)=6.486$, $p=.003$ and CS+: $F(3,18)=14.513$, $p<.001$). In addition, the Bonferroni-adjusted pairwise comparisons provided in SPSS GLM revealed significant differences in CS- startle measurements between position 1 and 4 ($p=.025$) and 2 and 4 ($p=.008$) and CS+ measurements between position 1 and 2 ($p=.049$), 1 and 3 ($p=.019$), and 1 and 4 ($p=.009$). Lastly, multiple paired t-tests comparing CS+ and CS- late startle measurements in each position condition were conducted. The only significant difference found between CS+ and CS- was in position 1 ($t(15)=3.062$, $p=.008$).

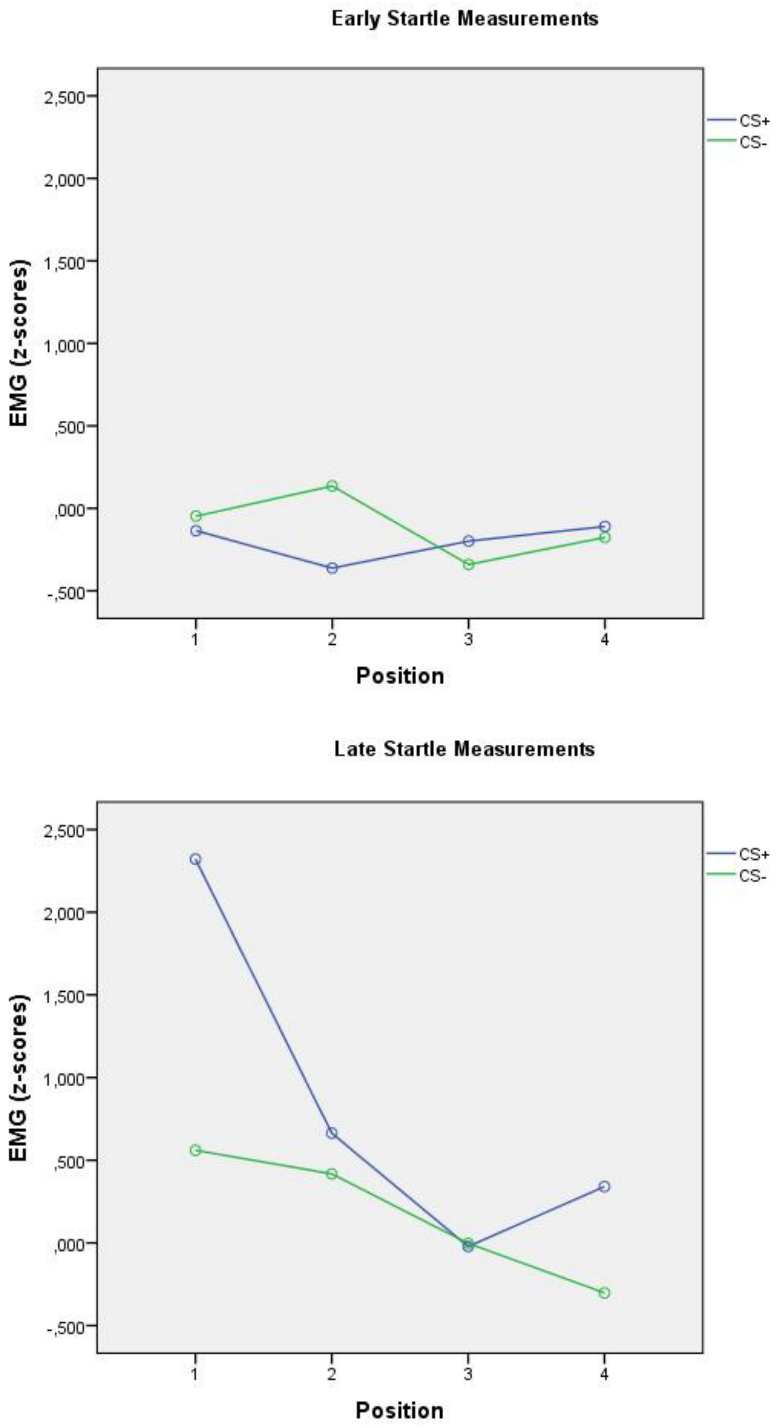

Fig. 4. Z-scores of early and late startle measurements in eight different conditions.

Discussion

Our pilot study investigated how distance to safety and the level of vigor necessary to avoid an aversive stimulus affect fear-potentiated startle. This was accomplished by having participants move a figure starting in one of four different locations into a safety area to avoid an electric shock and measuring fear-potentiated startle (FPS) both before and after the movement period.

It was hypothesized that decreasing distance to safety would be similar to increasing proximity to threat as described by McNaughton & Corr (2004). Shorter distance to safety (position 3) would result in amygdala activation and early startle potentiation, whereas longer distances (position 2) would lead to inhibition of early startle relative to short distances. When the figure was furthest away from the safety area (position 1), we expected startle to be potentiated due to the fact that it would be almost impossible to get to the safety area leading to high shock expectation. Late startle measurements were predicted to be greatest for position 1. Additionally, we hypothesized that there would be a significant difference between CS- and CS+ conditions for both early and late startle and that CS- startle measurements would not be affected by different starting positions.

Although there was an effect of position on all startle measurements (early and late combined), no effect on early startle was found. In addition, there was no difference between early startle CS+ and CS- conditions. These results contradict our main hypothesis, which stated that eye-blink startle would exhibit potentiation in close proximity and relative inhibition at higher distance. The fact that neither position nor CS had any effect on early startle suggests that the startle reflex was at a baseline level. The finding that late startle amplitudes were overall higher than early startle supports this notion. The decreased levels of early startle amplitude could have occurred due to a number of different reasons: firstly, the duration between startle probe and US could have been too large for the expectancy of shock to have any effect. There were 7.5 seconds between startle probe onset and shock onset as seen in Figure 3. Secondly, it is possible that anticipation of movement decreased startle indirectly by lowering shock expectancy. By focusing on the task at hand, participants may not anticipate the shock as much, which could lead to startle reflex inhibition. Thirdly, anticipation of movement could have inhibited startle directly. Previous research has shown that when a reaction time cue is anticipated, FPS is lower, which appears to be related to ignoring all task-relevant stimuli (Anthony, 1985; Löw et al., 2008). As participants anticipate the cue, they ignore the task-irrelevant startle stimulus and, therefore, startle is inhibited.

Although our study did not involve reaction time cues, there was a movement cue that signaled the start of the movement period, which could have highly similar effects.

Regardless of the reason for low startle amplitudes, the results make comparison of distance to safety and defensive distance to threat highly difficult. Even though it may seem logical to conclude from our results that the effects of distance to safety and proximity to threat are different from each other, it is important to realize that startle was the only measurement included in our study. Startle may be a good indicator of conditioned fear and amygdala activity; however, it also appears to be affected by other factors. Due to the nature of our avoidance paradigm, it was not possible to control for these factors making startle an imperfect measurement.

For late startle amplitude, main effects of position and CS as well as an interaction effect between the two were found. Our results revealed that CS+ late startle amplitudes were affected by the starting positions of the figure: starting position 1 was different from all the other positions, which is in line with our hypothesis. This result is not surprising as most of the participants (six out of nine) failed to move into the safety area from starting position 1 in 100% of the trials. Therefore, most participants received a shock shortly after late startle probe onset (1.5s) in CS+ position 1 trials, which is reflected in the high late startle amplitude. The fact that no differences were found between the other three positions can be explained by the fact that the majority of position 2, 3, and 4 trials were avoided successfully (for more detail, see Results section). Additionally, position 1 was the only starting location that resulted in a significant difference between CS+ and CS- conditions. Thus, the CS+/CS- distinction was only present when a shock was delivered.

Surprisingly, not only CS+, but also CS- late startle measurements were affected by the starting position of the figure: during late startle CS- trials, positions 1 and 2 resulted in higher startle amplitudes than position 4 (see also Fig. 4). One would expect startle amplitude to be the same for all four positions as there is no influence of the US that could potentiate startle in CS- trials: regardless of where the figure was located at the beginning of a trial, participants would not receive a shock. Therefore, expectancy of the US cannot be the reason for startle potentiation. Aside from startle amplitude, the CS- positions also differed with regard to participants' movement of the figure. Although no movement was necessary during CS- conditions, participants were still able to move to make the trials as comparable as possible to CS+ trials. The majority of subjects behaved similarly during CS- and CS+ trials. Therefore, the button presses necessary for figure movement could have increased general arousal of participants, which could have lead to startle potentiation, explaining the differences between position 1 & 4 and 2 & 4. This would be in line with the results of a control experiment by Xia & Baas (2014) in which a single button press potentiated subsequent startle measurements. Nevertheless, not all subjects decided to move the figure during CS- trials (for more detail, see Results section). As only nine different data sets were available and only three participants refrained from moving during CS- trials, a comparison between the different movement behaviors of participants was not possible. More research is necessary to investigate the possible link between movement and subsequent startle potentiation.

In the current pilot study, we used a novel fear conditioning avoidance paradigm in which distance to a safety area was varied. Recommendations on future research can be made on the basis of both the limitations as well as the results of the experiment. Firstly, contrary to our expectations, early startle measurements were not potentiated and CS- late startle amplitudes were affected by the starting position of the figure. These results exemplify that the acoustic startle reflex appears to be sensitive to anticipation of a cue and movement. More research is necessary to investigate all factors involved in the potentiation and inhibition of startle; researchers could then use FPS in the most effective way possible by creating paradigms eliminating or minimizing alternative explanations. Unfortunately, the nature of the avoidance paradigm used in the current experiment did not allow controlling for all the variables influencing startle. An example of this is that movement behavior could not be controlled. In all CS+ conditions, movement was necessary; however, some participants moved more than others. Moreover, some conditions required more movement, which may have influenced startle. During CS- conditions, there were clear individual differences in movement behavior that may have influenced late startle. In addition, the difficulty level of the different position conditions differed depending on individuals' abilities. The 'open' character of the task makes using physiological measures difficult; instead, the differences in movement behavior between participants suggest that the paradigm could be helpful in investigating individual differences in avoidance behavior or differences between groups such as patient populations. Additionally, the original paradigm of simply avoiding the shock could be made more complex, e.g. by adding a possible reward that the participant could receive by moving away from the safety area.

Conclusion

To our knowledge, this has been the first attempt to integrate different difficulties of avoidance into a fear conditioning paradigm. Startle appeared to be affected by a variety of factors illustrating how challenging integrating physiological measurements of fear into a relatively free task can be. Nonetheless, some unexpected findings demand further investigation. The paradigm could be useful in assessing individual or group differences in avoidance behavior.

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