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Cue conditioning using a virtual spider discriminates between high and low spider fearful individuals

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ABSTRACT

The fear conditioning paradigm is one of the most commonly used procedures to examine the etiology and treatment of anxiety disorders in laboratories. However, findings with this procedure often do not generalize to clinical settings. Virtual reality (VR) is a promising tool for improving the ecological and predictive validity of fear conditioning. The current study explored whether a classical differential cue conditioning paradigm with spider-fearful participants can be conducted in a VR-environment. Specifically, 25 spider-fearful and 25 non-fearful female students participated in a fear-conditioning experiment with a virtual spider as an unconditioned stimulus (US). The experiment took place in a virtual office in which participants viewed an avatar of themselves sitting at a desk. Conditioned stimuli (CS) were a blue (CS+; 100% reinforcement) and a green (CS-) light emitted by a desk lamp. Fear reactions were measured by fear ratings, skin conductance responses (SCR), and fear potentiated startle responses (FPS). Our results indicated stronger differential fear conditioning for spider-fearful participants than for non-fearful participants. Furthermore, we demonstrate that these results relate specifically to spider-fear, and not to general trait anxiety. We conclude that fear conditioning in VR is a promising tool to improve the validity of classical fear conditioning procedures.

1. Introduction

The etiology of fear and anxiety related disorders is mostly studied in the laboratory using the Pavlovian fear conditioning procedure (Mineka & Zinbarg, 2006). In a Pavlovian cue conditioning paradigm, a neutral conditioned stimulus (CS+; e.g., color of a light) is paired with an aversive unconditioned stimulus (US; e.g., electrotactile stimulation), resulting in fearful responses to the CS. In humans, often a differential conditioning procedure is often used, in which a second conditioned stimulus (CS-) is shown without the US (Lipp, 2006). These conditioning paradigms have been useful in studying the acquisition, expression, generalization, and inhibition of threat-related behavior, as conditioning is believed to be one of the underlying mechanisms of anxiety and stress disorders (Mineka & Oehlberg, 2008; Mineka & Zinbarg, 2006).

Despite its success, fear-conditioning research has several methodological limitations. That is, fear conditioning research is traditionally performed in laboratory settings, where simple and static stimuli are used (Parsons, 2015). These fear conditioning tasks usually represent a "strong situation", whereby encountered stimuli by an individual are unambiguous (Lissek, Pine, & Grillon, 2006). In strong situations, individuals show a similar (adaptive) response pattern, limiting variability across individuals. "Weak situations" characterized by ambiguity and uncertainty might provide better opportunity to discover individual differences, such as differences in fear learning patterns between patient populations and healthy controls (Beckers, Krypotos, Boddez, Effting, & Kindt, 2013). Although strong situations contribute to the high internal validity of experimental research (Scheveneels, Boddez, Vervliet, & Hermans, 2016), it has been argued that research in laboratories lack potentially important aspects of real world circumstances, resulting in low ecological validity (Parsons, 2015). Therefore, laboratory findings possibly cannot be generalized to people's everyday life (Parsons, 2015), and clinical practice (Scheveneels et al., 2016). For example, individual differences which are known risk factors for developing anxiety disorders, such as trait anxiety and the BDNF-val66met polymorphism, do not appear to modulate fear conditioning (Torrents-Rodas et al., 2012, 2013). Such findings are problematic for the idea that fear conditioning procedures allow examination of processes involved in the etiology of anxiety disorders (Beckers et al., 2013). Investigating fear conditioning by including real world settings can improve ecological and predictive validity, but at the cost of lower internal validity and experimental control, and higher economical costs (Parsons, 2015; Shiban, Reichenberger, Neumann, & Mühlberger, 2015).

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A promising new technology to improve the ecological and predictive validity of experimental models is virtual reality (VR) (Baas, Nugent, Lissek, Pine, & Grillon, 2004; Cuperus, Laken, Van Den Hout, & Engelhard, 2016; Dibbets & Fonteyne, 2015; Huff, Zeilinski, Fecteau, Brady, & LaBar, 2010; Shiban et al., 2015). VR uses head-mounted displays to present digitally recreated real-world environments and activities. Advances in VR-technology have improved the quality and ease of stimulus presentation, data collection, and processing, at a decreasing cost (Parsons, 2015). Hence, VR provides a feasible solution to use more ecologically valid stimuli without requiring large investments of time or money. Furthermore, in VR simulations, environment- and confounding variables can be controlled, providing experimental control, dynamic stimuli presentation, and better standardization (Parsons, 2015; Shiban et al., 2015). VR simulations can also give a sense of immersion (i.e., a strong feeling of being present in the virtual environment). People tend to think, behave, and feel as if they are in the virtual space, rather than in the real world (Kroes, Dunsmoor, Mackey, McClay, & Phelps, 2017). In fact, this feeling of 'presence' might influence fear perception (Juan & Pérez, 2009; Ling, Nefs, Morina, Heynderickx, & Brinkman, 2014), and can be enhanced by the presence of virtual hands (Peperkorn, Diemer, Alpers, & Mühlberger, 2016). Finally, VR-technology offers a set of tools to track motion- and eye-movement, facilitating the measurement of spontaneous behavior.

Despite its promising features to improve ecological and predictive validity, VR technology has not yet been extensively been used in fear conditioning research. Previous studies using virtually reality have shown that VR can be used to model acquisition, extinction, spontaneous recovery, and generalization of fear in cue conditioning (Baas et al., 2004; Ewald et al., 2014), social conditioning (Shiban et al., 2015), and context conditioning (Glotzbach, Ewald, Andreatta, Pauli, & Mühlberger, 2012; Huff et al., 2011). However, older VR-studies have typically used 3D-simulations presented on monitors, instead of a head-mounted display (HMD) which later became commercially available with the development of systems such as the Oculus Rift or the HTC Vive. The use of HMDs provide much higher levels of immersion and presence in the situation, which may be relevant for participants' fear levels (Bowman & McMahan, 2007; Juan & Pérez, 2009; Ling et al., 2014). Furthermore, most fear conditioning studies use pain signals (e.g., electro-tactile stimulation) as US, whereas a more naturalistic US could improve external validity of the conditioning procedure. In particular, disorder-relevant USs have been shown to facilitate fear conditioning, emphasizing the importance of disorder-relevant USs (Lissek et al., 2008). Lastly, differences in conditioning have been found for both individual characteristics and clinical samples (Lissek et al., 2008). Therefore, studies using sub-clinical participants might help in uncovering some of the underlying etiological and pathology-maintaining mechanisms in their associated clinical group. So far, only a few studies investigating fear conditioning in VR-environments used sub-clinical samples (Mosig et al., 2014) or participants at risk of developing anxiety disorders (Glotzbach-Schoon, Andreatta, et al., 2013; Glotzbach-Schoon, Tadda, et al., 2013; Shiban et al., 2015), which limits our understanding of the feasibility of using this technology with these groups.

In the current study, we wanted to investigate whether VR can be used to investigate conditioning processes in specific phobia. Specific phobia is one of the most common mental health disorders in Europe (Alonso et al., 2004; Bandelow & Michaelis, 2015). Worldwide, specific phobia has a lifetime prevalence of 7.4% in the general population, with higher rates found in females (9.8%) than in males (4.9%) (Wardenaar et al., 2017). Of specific phobias, fear of animals is one of the most common DSM-subtypes, of which spider phobia is among the most prevalent, and most studied (Miloff et al., 2016). In fact, it is particularly interesting to study fear conditioning and extinction processes in specific phobias because especially for specific phobias, conditioning experiences are believed to be causally related onset and maintenance of the disorder (Field, 2006; Schindler, Vriends, Margraf, & Stieglitz, 2016). However, studies with patients often fail to find evidence for the involvement of conditioning processes in specific phobia (Menzies & Clarke, 1995; Rachman, 1977). This may be due to memory biases and forgetting in studies with patients, who often already suffer from their condition for many years. Therefore, studies with participants reporting sub-clinical levels of fear can be particularly informative to determine whether conditioning processes are different prior to the onset of a specific phobia. Finally, for spider phobia exposure therapy in a VR-environment is already available and effective (Garcia-Palacios, Hoffman, Carlin, Furness, & Botella, 2002; Michaliszyn, Marchand, Bouchard, Martel, & Poirier-Bisson, 2010; Opriş et al., 2012; Shiban, Pauli, & Mühlberger, 2013). This suggests that spider-related fear can be safely studied in a VR-simulation.

Given these features of specific phobia, and particularly spider phobia, the aim of the current study was to explore whether VR technology could be used to create a more ecologically valid version of the fear conditioning procedure for participants with sub-clinical levels of fear for spiders. Therefore, a virtual office environment was used whereby participants viewed an avatar of themselves sitting at a desk. A female avatar was chosen, because spider phobia is more common among women (Fredrikson, Annas, Fischer, & Wik, 1996). We used a VR spider as an ecologically valid and disorder-relevant US, which was paired with a neutral CS (i.e., a blue light emitted by a desk lamp; CS +). Following the typical differential fear conditioning procedure with humans, a second CS (i.e., a green light; CS-) was not paired with the virtual spider. As a control condition, we included a group of participants without fear for spiders. Aversive responses were measured by subjective fear ratings, skin conductance response, and the startle response. We hypothesized that a more ecologically valid US could be used to condition participants in a VR environment, specifically for participants for who this US is likely unpleasant and aversive.

2. Method

2.1. Pilot study

An explorative pilot study was conducted to determine optimal spider presentation parameters for provoking fearful responses in spider fearful participants, following a similar procedure and the same setting (i.e., a virtual office) as the main study. In total, six participants took part in the pilot (2 men; 4 women), with an average score of 61.33 (SD = 27.78) on the Fear of Spider Questionnaire (FSQ). Two female participants dropped out beforehand, due to too little and too much fear of spiders (FSQ scores of 38 and 110, respectively). The pilot consisted of 10 spider presentations (duration: 15 s, inter-trial interval: 10 s) that were presented in clusters of three, after which participants were asked to select the most fear-inducing spider ("Which spider did you find most annoying to see?", available options: first, second or third spider). In each cluster, different spider characteristics were varied (e.g. size, movement speed, or both). The last trial contained a small technical glitch in the spider's movement, to assess whether this influenced the participant's experience. Using a short structured interview at the end of the pilot, we found that spiders intermediate in size and that varied in trajectory and movement speed were perceived as the most unpleasant in this sample.

2.2. Pre-registration main study

The sample size determination, design, procedure, and data analyses steps of the main study were pre-registered on the Open Science

Framework prior to finalizing the data collection (https://osf.io/wakqv/).

2.3. Participants

Eligibility requirements for this study included an age restriction of 18–50 years. Participants were also required to have normal (or by lenses corrected) eyesight and vision, and normal (or corrected) hearing. Furthermore, participants with a current or relevant history of a psychiatric or neurological condition, or who had received psychological treatment in the past two years were excluded from this study. Finally, due to the use of a female avatar in the virtual reality simulation, only female participants were able to participate. Participants were recruited through on campus advertisement at Utrecht University, the university's social media, and experiment recruitment websites. Participants received 8 euro for compensation or course credit as part of the university's psychology bachelor curriculum. The Ethics committee of the Faculty of Social and Behavioral Sciences approved the study (FETC16-054).

A total of 129 participants initially responded, of which 38 (29.46%) failed to reply or cancelled, and 41 (31.78%) were excluded due to exclusion criteria or insufficient fear of spiders. A sample of 50 female participants completed the experiment. However, two participants from the spider fearful group could not verbalize the contingencies in the experiment and were therefore excluded from the analyses (see our pre-registration). Demographic information for the final sample included in the analyses is provided in Table 1.

2.4. Materials and stimuli

In the VR environment, participants were seated at a table in a virtual office (which was different from the physical laboratory) and were able to see a VR avatar (i.e., a woman seated at a table, with her hands resting on the table) from a first-person point of view (Fig. 1). Participants were instructed to sit still, put their arms on the table, and to not move their arms in order to create the illusion that the avatars' arms were their own. To further enhance the feeling of immersion in the VR environment, the visual field of the HMD (110°, stereoscopic vision) followed the head movements of the participants. The CS was a desk light that was placed on the far-left side of the table. A blue emitted light was followed by the presentation of the spider (i.e., the reinforced conditioned stimulus, CS+), and a green light was not followed by the spider (i.e., the unreinforced conditioned stimulus, CS-; Fig. 2). The US was a virtual spider with an exotic appearance. Based on the pilot, the size of the spider varied per trial between approximately 4 to 8 cm in diameter. Furthermore, the spider varied in movement speed within-, and between trials (between 0.3 and 0.8 m/s), to make its behavior unpredictable (Grillon, Baas, Cornwell, & Johnson, 2006). After every reinforced CS + offset, the spider appeared in the visual field from behind the desk light, and made one of five different pre-programmed movements: walking over the left side of the table, walking to the right end of the table, approaching the hands of the avatar and walking away to the right end of the table, walking towards the right end of the table and turning back towards the right arm, or walking over the hands of the avatar to the right side of the table.

The virtual reality simulation was custom-designed in Unity (5.5.3) and was presented with the Oculus Rift VR headset (Version CV1; Oculus, USA) Oculus Rift App (Version 1.19.0.456194). The experiment was run on a Window 10 desktop computer with a quadcore Intel Core i7-6700K (4.00 GHz), 16 GB RAM, and a NVIDIA GeForce GTX 1060 6 GB.

Fig. 1. Screenshots of the virtual reality environment and stimuli: A 2D depiction of the virtual office (top left), the reinforced conditioned stimulus (top right), the unreinforced conditioned stimulus (bottom left), and the smallest and largest virtual spider (bottom right). Participants always saw the room from a first-person perspective.

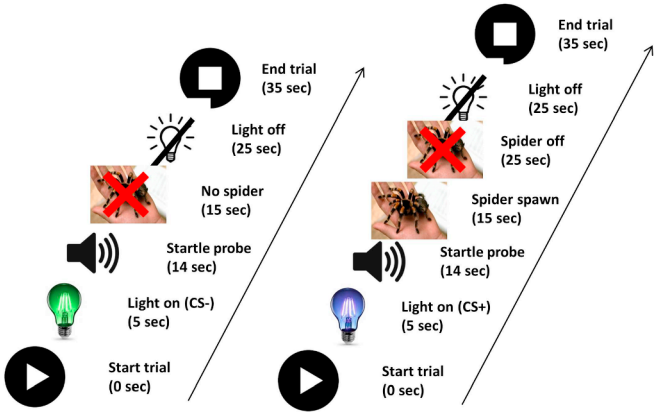

Fig. 2. Trial sequence and duration times per trial for CS- (left) and CS+ (right) trials.

Table 1
Demographic information, FSQ scores and STAI-DY2 scores for the spider-fearful and non-fearful group.

	Spider non-fearful Group	Spider fearful Group	Difference
N	25	23	
Age			
Mean age in years (SD)	21.32 (1.73)	21.52 (2.45)	$F(1, 46) < 1$
Actual range (min-max)	19–25	18–26	
FSQ score			
Mean (SD)	14.92 (13.42)	73.09 (15.86)	$F(1, 46) = 189.13^{*}$
Actual range	0–45	44–107	
STAI-DY2 score			
Mean (SD)	33.88 (7.51)	32.83 (6.31)	$F(1, 46) < 1$
Actual range	23–55	23–47	

Note: $^{*}p < .001$, Cohen's $d = 3.96$.

2.5. Questionnaires

Fear of spiders was measured with the FSQ (Muris & Merckelbach, 1996; Szymanski & O'Donohue, 1995). The FSQ is a self-report questionnaire that consists of 18-items scored on a 0-7-point scale. The FSQ has good internal consistency ($\alpha = 0.92$ and split-half reliability) ($\alpha = 0.89$) (Szymanski & O'Donohue, 1995). Muris and Merckelbach (1996) found a mean score of 89.1 ($SD = 19.6$) in their spider phobic sample. However, because we did not want to include spider phobic participants in our study and based on the pilot study, a score of 55 or

higher was estimated to be a sufficiently high level of fear of spiders to perceive the VR spider as unpleasant. For the control group, a FSQ score of ≤ 45 was considered non-fearful.

Furthermore, the State-Trait Anxiety Inventory DY-2 was used to measure trait anxiety (STAI-DY-2 version; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983; Dutch translation: van der Ploeg, Defares, & Spielberger, 2000). The STAI-DY-2 is a self-report questionnaire consisting of 20-items, each rated on a 1–4 scale, measuring trait anxiety. The STAI-DY-2 has good internal consistency ($\alpha = 0.91$ for female students) (van der Ploeg, 1982). Trait anxiety refers to a relatively stable construct of fear disposition, i.e. the tendency to react to perceived threatening situations with a heightened intensity of state anxiety (van der Ploeg, 1982). The questionnaire was administered to control for possible differences in general anxiety between the two groups as general anxiety may affect fear conditioning (Lissek et al., 2005).

2.6. Outcome measures

2.6.1. Subjective fear ratings

Subjective fear ratings were assessed at the beginning of each trial, after the presentation of the CSs, and before the startle probe administration. Participants were verbally asked to rate the question "How afraid are you at this moment?" on a scale ranging from 0 (not fearful) to 100 (very fearful).

2.6.2. Physiological fear responses

The physiological fear responses to the CS presentations were measured with the skin conductance response (SCR), as well as the fear potentiated startle (FPS) response. SCR and FPS were registered with a BioSemi System (Amsterdam, Netherlands). SCRs were measured using two BioSemi GSR electrodes (0.8 cm diameter) attached to the thenar and hypothenar eminences of the left hand, which was cleaned with tap water (Fowles et al., 2012). FPS was measured with two BioSemi EMG electrodes (0.4 cm diameter) attached below the left eye (Blumenthal et al., 2005).

2.6.3. Post-experimental interview

A brief standardized interview was conducted to measure contingency awareness. Participants were asked to rate their overall spider expectancy for each CS on a scale from 0 to 100, and which color light predicted the spider's presence. Participants were also asked to rate the unpleasantness of seeing the spider on a scale of 0–100.

2.7. Procedure

Before the study, participants filled out an online form containing a screening questionnaire and the FSQ. After arrival in the lab, participants received oral and written information about the study, after which they signed an informed consent, and filled out the STAI, and FSQ (2nd administration; this was included to confirm the scores obtained with the online FSQ administration). Next, participants' skin was cleaned (with Nuprep gel), electrodes were placed, and the headset was put on. Participants were instructed to monitor which color light predicted the spider and were told that they had to indicate their level of fear at the beginning of each trial. During the experiment, participants sat behind a desk, with their hands placed on the table matching those of the avatar. The experiment consisted of three phases: habituation, acquisition, and extinction. The habituation phase was preceded by a trial with a neutral desk light, to provide a moment for the participants to get acquainted with the VR-environment.

In the habituation phase, each CS was presented twice, without the presentation of the spider. During the acquisition phase, each CS was presented five times. In this phase, the CS+ was followed by the presentation of the spider each time (100% reinforcement). In the extinction phase, each CS was presented eight times without reinforcement. In each phase, the order of CS presentations was randomized and counterbalanced between groups. CSs were presented on the screen for 20 s and were followed by a 15 s inter-trial interval. In case of a reinforced CS + presentation, the US (spider) was presented during the last 10 s of the CS presentation. At CS onset, participants were verbally asked to indicate their level of fear by the experimenter (see Section 2.6.1). Nine seconds after each CS onset, a startle probe (50 ms white noise; 85 dB) was administered. A schematic overview of the trial procedure and stimulus timing is provided in Fig. 2.

2.8. Data preprocessing and analysis

2.8.1. Data reduction

Two participants were excluded from all analyses because they could not specify the contingency between the color of the desk light and the presentation of the spider, as measured with a post-experimental interview (see section 2.3). Because a 100% contingency between the CS+ and US was used in the acquisition phase, these participants were considered to be inattentive (Leer, Engelhard, Dibbets, & van den Hout, 2013). Furthermore, four participants' physiological data were missing because electrodes disconnected during the experiment, which resulted in a final sample of 48 participants for the subjective analysis and 44 participants for the FPS and SCR analyses. For one participant, one subjective rating value was missing. This value was replaced by the average of the remaining trials of that phase.

2.8.2. Skin conductance response

The skin conductance signal was downsampled to 10 Hz using BrainVision Analyzer software. SCRs were then scored by subtracting a mean baseline value (the 2 s preceding CS onset) from the highest response value in the 9 s time windows after CS onset (Pineles, Orr, & Orr, 2009). A minimum criterion of 0.02 μS was applied for the SCRs. To account for inter-individual differences in responsiveness, SCR values were range corrected (using the maximal response to the CSs). Furthermore, to normalize the distribution of the data, SCR values were square root transformed (Dawson, Schell, & Filion, 2007).

2.8.3. Startle response

The electromyography signal of the fear potentiated startle was filtered (28–500 Hz), rectified, and smoothed (using a 15.9 Hz low-pass filter) with BrainVision Analyzer software. Startle magnitude was automatically scored by subtracting the baseline value (time window: 30 ms before probe onset to 20 ms after probe onset) from the highest peak value in the 150–250 ms time window after startle probe onset. Due to the VR software, a consistent delay of approximately 100 ms was introduced between the parallel port triggers and the startle probe administration. This delay was taken into account for our scoring window determination of the EMG signal (i.e., the actual scoring window after startle probe onset was 50–150 ms). These values were then T-transformed using each participants' individual mean and standard deviation (Blumenthal et al., 2005).

2.8.4. Data analyses

The different measures of fear (subjective fear ratings, SCR, FPS) were analyzed with several repeated measures ANOVA's for each of the different phases in the experiment. Age, FSQ scores, STAI-DY-2 scores, and spider unpleasantness ratings were analyzed with a one-way between-subjects ANOVA with Group (spider fearful group and spider non-fearful group) as a factor. For all statistical analyses an alpha level of .05 was applied. Analyses were carried in SPSS version 24. Violations

of the sphericity assumption were corrected using Greenhouse-Geisser corrections.

3. Results

3.1. Spider unpleasantness ratings

The one-way ANOVA for spider unpleasantness ratings showed a significant main effect of Group, $F(1, 46) = 87.73$, $p < .001$, $\eta_p^2 = 0.66$, indicating that participants in the spider fearful group found the spider more unpleasant ($M = 69.78$, $SD = 19.39$, range = 0–100) than participants in the spider non-fearful group ($M = 16.34$, $SD = 20.07$, range = 0–70).

3.2. Subjective fear ratings

Subjective fear ratings during the habituation phase were analyzed with a repeated measures ANOVA with factors CS type (within-subjects; CS+ and CS-), Trial (within-subjects; first trial and second trial) and Group (between-subjects; spider fearful group and spider non-fearful group) showed no significant results, F-values < 1.60, indicating no differences between groups, trial, and CS type.

For the acquisition phase, a repeated measures ANOVA with factors CS type (CS+, CS-), Trial (trial 1 to 5) and Group (spider fearful group, spider non-fearful group) showed main effects for CS type, $F(1, 46) = 37.68$, $p < .001$, $\eta_p^2 = 0.45$, and Trial, $F(2.10, 96.41) = 12.01$, $p < .001$, $\eta_p^2 = 0.21$, which were modulated by a significant interaction CS type and Trial, $F(2.37, 108.94) = 14.98$, $p < .001$, $\eta_p^2 = 0.25$. A significant main effect for Group was also found, $F(1, 46) = 31.44$, $p < .001$, $\eta_p^2 = 0.41$. Furthermore, there was a significant interaction between CS type and Group, $F(1, 46) = 10.38$, $p = .002$, $\eta_p^2 = 0.18$, and Trial and Group, $F(2.10, 96.41) = 13.28$, $p < .001$, $\eta_p^2 = 0.22$. Lastly, there was a significant three-way interaction between CS type, Trial and Group, $F(2.37, 108.94) = 5.46$, $p = .003$, $\eta_p^2 = 0.11$. Additional post-hoc t-tests showed a significant difference between the CS+ ($M = 12.60$, $SD = 16.12$) and the CS- ($M = 5.48$, $SD = 8.21$) at the last acquisition trial for the non-fearful group, $t(24) = 3.19$, $p = .004$, $d_z = 0.64$, and for the spider fearful group (CS+: $M = 51.15$, $SD = 22.89$; CS-: $M = 29.13$, $SD = 25.88$), $t(22) = 5.14$, $p < .001$ $d_z = 1.07$. These results indicate a differential fear acquisition for the CS + compared to the CS-, with the conditioned differential fear ratings being larger for the spider fearful group than the non-fearful group (see Fig. 3).

For the extinction phase, a repeated measures ANOVA with factors CS type (CS+, CS-), Trial (trial 1 to 8) and Group (spider fearful group, spider non-fearful group) showed main effects for CS type, $F(1, 46) = 49.78$, $p < .001$, $\eta_p^2 = 0.520$, and Trial, $F(1.56, 71.61) = 37.53$, $p < .001$, $\eta_p^2 = 0.449$, which were also modulated by a significant interaction between CS type and Trial, $F(2.17, 99.79) = 12.43$, $p < .001$, $\eta_p^2 = 0.21$. A significant main effect for Group was found, $F(1, 46) = 25.12$, $p < .001$, $\eta_p^2 = 0.35$, as well as an interaction between CS type and Group, $F(1, 46) = 20.63$, $p < .001$, $\eta_p^2 = 0.310$, and Trial and Group, $F(1.56, 7.61) = 18.28$, $p < .001$, $\eta_p^2 = 0.284$. Lastly, there was a significant three-way interaction between CS type, Trial and Group, $F(2.17, 99.79) = 5.35$, $p = .005$, $\eta_p^2 = 0.104$. The results indicate that fear ratings were overall higher for the CS + than for the CS-, and decreased throughout the extinction phase, with a larger decrease for the spider-fearful group than the non-fearful group. However, t-tests showed that the difference between the CS+ ($M = 20.57$, $SD = 18.90$) and CS- ($M = 8.83$, $SD = 11.61$) remained significant for the spider fearful group at the last extinction trial, $t(22) = 3.62$, $p = .002$, $d_z = 0.75$. The difference between the CS+ ($M = 5.04$, $SD = 8.67$) and CS- ($M = 3.16$, $SD = 7.61$) also remained significant for the non-fearful group, $t(24) = 2.75$, $p = .011$, $d_z = 0.55$.

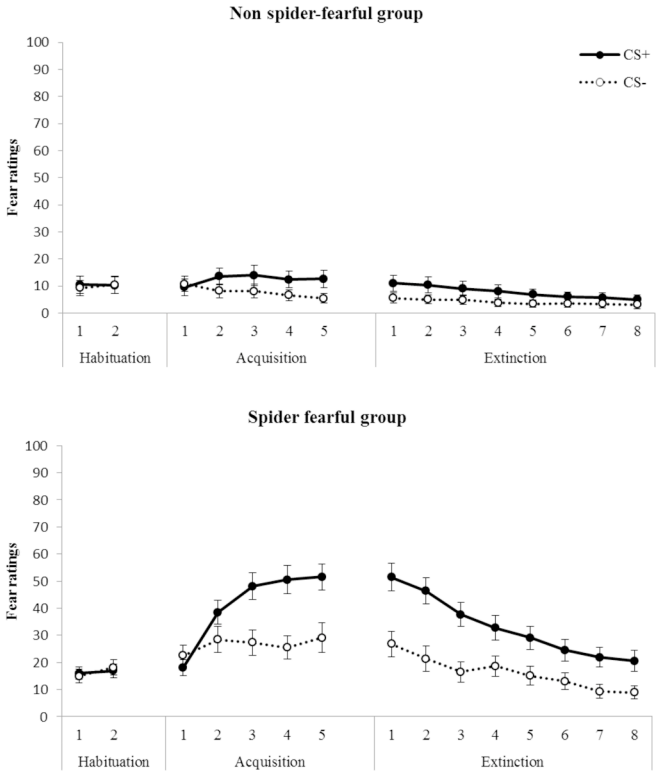

Fig. 3. Subjective fear ratings for the spider non-fearful and spider fearful group. Error bars reflect standard error.

3.3. Skin conductance responses

SCR-responses during the habituation phase were analyzed with a repeated measures ANOVA with factors CS type (within-subjects; CS+ and CS-), Trial (within-subjects; first trial and second trial) and Group (between-subjects; spider fearful group and spider non-fearful group) was used. No significant effects were found, all F-values < 1.50, p-values > .240, η_p^2's < 0.04, indicating no differences between groups or CS type before the acquisition phase.

For the acquisition phase, a repeated measures ANOVA with factors CS type (CS+, CS-), Trial (trial 1 to 5) and Group (spider fearful group, spider non-fearful group) showed a main effect for CS type, $F(1, 42) = 24.52$, $p < .001$, $\eta_p^2 = 0.37$, but not for Trial, $F(4, 168) = 0.46$, $p = .763$, $\eta_p^2 = 0.01$. However, a significant two-way interaction was found between CS type and Trial, $F(4, 168) = 3.42$, $p = .010$, $\eta_p^2 = 0.75$, indicating differential fear learning. No significant interaction was found between CS, Trial and Group, $F(4, 168) = 0.62$, $p = .650$, $\eta_p^2 = 0.02$. All other effects were non-significant, F-values < 2.00, p-values > .110, η_p^2's < 0.05. Exploratory follow-up t-tests showed no significant difference between the CS+ ($M = 0.44$, $SD = 0.36$) and CS- ($M = 0.39$, $SD = 0.35$) at the last acquisition trial for the non-fearful group, $t(22) = 0.86$, $p = .398$, $d_z = 0.18$. However, there was a significant difference between the CS+ ($M = 0.53$, $SD = 0.31$) and CS- ($M = 0.30$, $SD = 0.26$) for the spider fearful group, $t(20) = 2.89$, $p = .009$, $d_z = 0.63$ (see Fig. 4).

For the extinction phase, a repeated measures ANOVA with factors CS type (CS+, CS-), Trial (trial 1 to 8) and Group (spider fearful group, spider non-fearful group) showed a significant interaction effect between Trial and Group, $F(7, 294) = 2.35$, $p = .024$, $\eta_p^2 = 0.05$, which can be explained by a decrease of SCRs for both CS types for the non-fearful group, and an increase of SCRs for both CS types the spider-fearful group. No main effects were found for CS type, $F(1, 42) = 1.75$, $p = .193$, $\eta_p^2 = 0.04$, Trial, $F(7, 294) = 0.68$, $p = .686$, $\eta_p^2 = 0.02$, or Group, $F(1, 42) = 3.05$, $p = .088$, $\eta_p^2 = 0.068$. No two-way

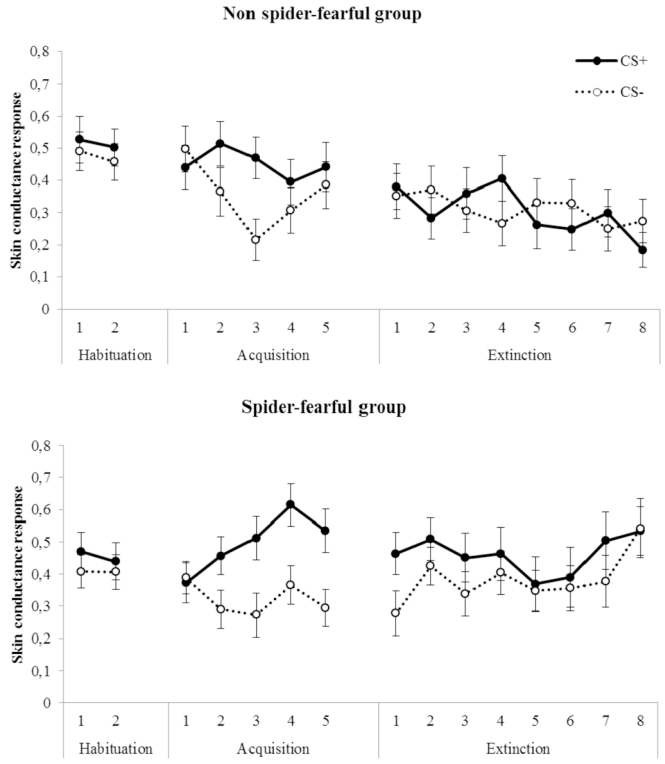

Fig. 4. Range corrected and square root transformed skin conductance responses for the spider non-fearful and spider fearful groups. Error bars reflect standard error.

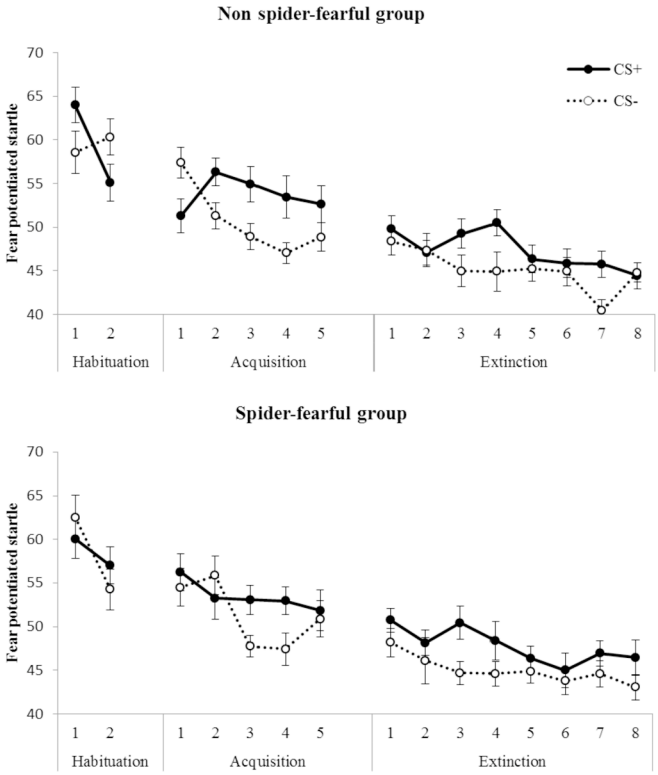

Fig. 5. T-transformed startle responses for the spider non-fearful and spider fearful groups. Error bars reflect standard error.

interactions were found between CS type and Trial, $F(7, 294) = 1.53$, $p = .158$, $\eta_p^2 = 0.04$, or CS type and Group, $F(1, 42) = 2.56$, $p = .117$, $\eta_p^2 = 0.058$, and no three-way interaction was found, $F(7, 294) = 0.54$, $p = .808$, $\eta_p^2 = 0.013$. Exploratory follow-up t-tests for the last trial of the extinction phase showed no significant difference between the CS+ ($M = 0.18$, $SD = 0.26$) and CS- ($M = 0.27$, $SD = 0.33$) for the non-fearful group, $t(22) = -1.27$, $p = .218$, $d_z = -0.26$, and no significant difference between the CS+ ($M = 0.53$, $SD = 0.34$) and CS- ($M = 0.54$, $SD = 0.42$) for the spider fearful group, $t(20) = -0.87$, $p = .931$, $d_z = -0.19$, indicating successful extinction.

3.4. Fear potentiated startle

FPS response during the habituation phase were analyzed with a repeated measures ANOVA with factors CS type (within-subjects; CS+ and CS-), Trial (within-subjects; first trial and second trial) and Group (between-subjects; spider fearful group and spider non-fearful group) showed no main effect for CS type, $F < 1.00$. There was a significant main effect for Trial, $F(1, 42) = 8.06$, $p = .007$, $\eta_p^2 = 0.16$, with overall lower responses during the second trial compared to the first trial. The three-way interaction between CS, Trial and Group was also significant, $F(1, 42) = 5.63$, $p = .022$, $\eta_p^2 = 0.12$, which can be explained by higher initial responses to the opposite CS for each group, with a stronger habituation for the respective CS during the second trial. Note that this difference cannot be due to randomization, as each randomization was counterbalanced per group. No other significant effects were found, all F-values < 1.00, p-values $> .400$, η_p^2's < 0.02.

For the acquisition phase, a repeated measures ANOVA with factors CS type (CS+, CS-), Trial (trial 1 to 5) and Group (spider fearful group, spider non-fearful group) showed a main effect for CS type, $F(1, 42) = 7.03$, $p = .011$, $\eta_p^2 = 0.14$, a main effect of trial, $F(4, 168) = 5.03$, $p = .001$, $\eta_p^2 = 0.11$, and an interaction between CS type and trial, $F(4, 168) = 3.34$, $p = .012$, $\eta_p^2 = 0.07$. These results reflect higher startle responses for the CS + than for the CS-, which became more pronounced towards the end of the acquisition phase (see Fig. 5). However, no interaction was found between CS, Trial and Group, $F(4, 168) = 2.28$, $p = .063$, $\eta_p^2 = 0.05$, and no other significant effects were found, all F-values < 1.00, p-values $> .650$, η_p^2's < 0.01. Exploratory follow-up t-tests at the last acquisition trial showed no significant difference between the CS+ ($M = 52.65$, $SD = 10.19$) and CS- ($M = 48.83$, $SD = 7.83$) for the non-fearful group, $t(22) = 1.26$, $p = .220$, $d_z = 0.26$, and no significant difference between the CS+ ($M = 51.87$, $SD = 10.54$) and CS- ($M = 50.89$, $SD = 9.36$) for the spider fearful group, $t(20) = 0.35$, $p = .731$, $d_z = 0.08$.

Regarding the extinction phase, a repeated measures ANOVA with factors CS type (CS+, CS-), Trial (trial 1 to 8) and Group (spider fearful group, spider non-fearful group) showed a main effect for CS type, $F(1, 42) = 18.31$, $p < .001$, $\eta_p^2 = 0.30$, and Trial, $F(7, 294) = 4.03$, $p < .001$, $\eta_p^2 = 0.09$, with a higher startle response to the CS + than the CS- and decreasing startle responses over time. No interaction was found between CS, Trial and Group, $F(5.35, 224.63) = 0.46$, $p = .836$, $\eta_p^2 = 0.01$. No other significant main or interaction effects were found, all F-values < 1.10, p-values $> .350$, η_p^2's < 0.03. Exploratory follow-up t-tests for the last trial of the extinction phase showed no significant difference between the CS+ ($M = 44.44$, $SD = 7.16$) and CS- ($M = 44.81$, $SD = 5.23$) for the non-fearful group, $t(22) = -0.23$, $p = .820$, $d_z = 0.05$, and no significant difference between the CS+ ($M = 46.45$, $SD = 9.46$) and CS- ($M = 43.05$, $SD = 6.68$) for the spider fearful group, $t(20) = 1.77$, $p = .092$, $d_z = 0.39$.

3.5. Regression analyses

In addition to the categorical analyses reported above, we conducted regression analyses to relate spider unpleasantness ratings, fear acquisition and fear extinction with FSQ and STAI-DY-2 scores of the full sample (i.e., participants from both the spider fearful and spider non-fearful group). For the spider unpleasantness ratings, a significant linear relationship was found with the FSQ scores, $\beta = 0.859$, $F(1,$

46) = 114.46, $p < .001$, with a Pearson's correlation coefficient of $r = 0.85$. In contrast, STAI-DY-2 scores were not significantly related to spider unpleasantness ratings, $\beta = -0.265$, $F(1, 46) = 0.14$, $p = .710$, Pearson's $r = -0.06$.

For the results of the acquisition phase, an acquisition index was calculated by subtracting the fear responses (subjective fear ratings, SCRs, and FPS, respectively) to the CS- from the fear responses to the CS + on the last trial of the acquisition phase. This acquisition index was significantly related to the FSQ scores for the subjective fear ratings, $\beta = 0.280$, $F(1, 46) = 15.92$, $p < .001$, Pearson's $r = 0.51$, but not for SCRs, $\beta = 0.003$, $F(1, 42) = 3.48$, $p = .069$, Pearson's $r = 0.28$, or FPS, $\beta = -0.258$, $F(1, 42) = 0.48$, $p = .493$, Pearson's $r = -0.11$. It was not significantly related to STAI-DY-2 scores for either subjective fear ratings, $\beta = -0.248$, $F(1, 46) = 0.42$, $p = .521$, Pearson's $r = -0.10$, or SCRs, $\beta = -0.002$, $F(1, 42) = 0.06$, $p = .815$, Pearson's $r = -0.04$. However, STAI-DY-2 scores were significantly related to the acquisition index for FPS, $\beta = -0.172$, $F(1, 42) = 5.17$, $p = .028$, Pearson's $r = -0.33$. However, this latter linear relationship was in the opposite direction than what was expected (i.e., higher trait anxiety is related to a smaller acquisition index rather than a larger acquisition index).

Likewise, for the results of the extinction phase, an extinction index was calculated by subtracting the fear responses (subjective fear ratings, SCRs, and FPS, respectively) to the CS- from the fear responses to the CS + on the first trial of the extinction phase and further subtracting this difference score from the difference between the CS+ and CS- on the last trial of the extinction phase (i.e., a higher extinction index indicates greater extinction). This extinction index was significantly related to the FSQ scores for the subjective fear ratings, $\beta = 0.153$, $F(1, 46) = 6.77$, $p = .012$, Pearson's $r = 0.36$, but not for SCRs, $\beta = 0.002$, $F(1, 42) = 0.73$, $p = .397$, Pearson's $r = 0.13$, or FPS, $\beta = -0.245$, $F(1, 42) = 0.35$, $p = .556$, Pearson's $r = -0.09$. The extinction index was not significantly related to STAI-DY-2 scores for subjective fear ratings, $\beta = -0.001$, $F(1, 46) = 0.00$, $p = .996$, Pearson's $r < -0.01$, SCRs, $\beta = -0.020$, $F(1, 42) = 2.91$, $p = .096$, Pearson's $r = -0.25$, or FPS, $\beta = -0.044$, $F(1, 42) = 0.25$, $p = .620$, Pearson's $r = -0.08$.

4. Discussion

In the present study, we investigated differential fear conditioning and extinction using an ecological and disorder-relevant US in VR. We found that spider fearful participants experienced confrontations with a VR spider as unpleasant, and found the VR spider significantly more unpleasant than spider non-fearful participants. Furthermore, we found differential fear acquisition on subjective fear ratings and SCRs, especially for the spider fearful group, which supports the idea that a visual US in VR can be used successfully for cue conditioning. Acquisition of conditioned fear measured with FPS responses was less clear due to absence of significant differences between the CS+ and CS- at the end of the acquisition phase for both groups. Furthermore, we found that conditioned differential fear ratings and SCRs decreased in an extinction phase. Finally, regression analyses indicated that these results were specific to spider-fearfulness as measured with the FSQ, and could not be explained by general trait anxiety levels as measured with the STAI-DY-2.

The findings of this study support the idea that fear conditioning in VR can be used to investigate conditioning and extinction processes in a sub-clinical group of spider-fearful individuals using an ecologically valid US. This is promising, because currently most conditioning procedures evoke uniform fear responses in participants high and low at risk for developing anxiety disorders and are often not moderated by factors known to be relevant in psychopathology. VR provides a promising tool to overcome these limitations of the fear conditioning procedure. Our results corroborate this idea by demonstrating that a VR version of the fear conditioning procedure distinguished between participants at risk and not at risk for developing spider phobia (i.e., convergent validity) while using a US that was perceived as unpleasant specifically for the spider fearful group (i.e., ecological validity). We also found typical fear acquisition and extinction curves using measures that are commonly used in fear conditioning research, thus maintaining the strong internal validity of fear conditioning research.

One surprising finding of our study was that fear acquisition was not very pronounced with FPS. A possible explanation for this finding is that the VR headset interfered with the measurement of the startle reflex. While we took caution to fix the EMG electrodes under participants' left eye and the ground electrodes on participants' forehead, putting on and wearing the VR headset did often put pressure on the EMG electrodes and the cables, sometimes shifting the electrodes slightly and even detaching them. Though we think that EMG measurement is possible while wearing a VR headset, it does interfere with optimal placement and attachment of EMG electrodes. Another surprising observation in our study was that the fear measures also showed some acquisition for the spider non-fearful group. This observation might be explained by the fact that also in this control group some people found the VR spider quite unpleasant (up to an unpleasantness rating of 70). On the other hand, for some participants in the spider-fearful group, the VR spider was not unpleasant at all (i.e., an unpleasantness rating of 0). Participants' verbal reports after the experiment indicated that not all participants experienced the VR simulation as realistic. Some participants in both groups found the VR environment video game-like and found the spider too unrealistic in size and appearance. Nonetheless, the majority of the spider fearful participants consistently found the VR spider unpleasant, whereas the majority of the spider non-fearful participants found the VR spider only mildly or not at all unpleasant.

In future studies, VR may be used to further improve the validity of the fear conditioning procedure in several ways. First, whereas this study used a naturalistic US, a more ecological valid CS can be used as well. Meaningful stimulus-stimulus (i.e., CS-US) matches can further facilitate acquisition, and impede extinction processes (Lonsdorf et al., 2017). Examples of such ecological valid CSs are stimuli that in real life are associated with the presence of spiders, such as spider webs, trees and bathroom sinks. Secondly, VR provides the opportunity to create realistic and dynamic contextual environments. This is especially interesting for investigating extinction-related processes which are known to be highly context-specific (Bouton, 2002). In VR, immersive environments can be created that are more realistic than the contexts that are typically used in the lab (e.g., change of the experiment room, changes in the background of the computer screen). Such realistic VR contexts may strengthen the contextual control of extinction memories and would thus allow us to study these processes in a more realistic fashion. Thirdly, VR offers the possibility to improve the integrated measurement of emotions by including approach-avoidance behavior components (Scheveneels et al., 2016). According to emotion theory, emotions (e.g., fear) are based on three dimensions, i.e., subjective experience, physiological activity, and approach-avoidance behavior (Beckers et al., 2013). Even though these dimensions are considered equally important, most fear conditioning studies neglect behavioral measurements. VR-technology lends several tools, such as VR-interactive controllers, to portray and measure spontaneous behavioral movements in a controlled manner. These tools have only recently become commercially available and offer new opportunities for further research. Finally, fear conditioning research using VR provides the opportunity to create a stronger link with treatment, particularly VR exposure treatment. For instance, manipulations known to be effective in VR exposure treatment may be translated to a VR extinction setting to establish whether both procedures result in comparably effects and rely on similar mechanism. Vice versa, manipulations found to be effective in fear extinction studies can be more easily translated to a treatment context.

Several limitations of the present study should be considered. One

important limitation is that we did not investigate the predictive validity of our adapted fear conditioning procedure. Scheveneels et al. (2016) argue that increasing the predictive validity is the most important goal of experimental models of psychopathology. Indeed, much suffering and economic costs could be avoided when we could precisely identify people at risk of developing anxiety disorders or accurately identify patients who will be responsive to treatment. While we think that our adjusted VR procedure definitely takes a step into this direction by improving other validity criteria, future studies should also investigate whether VR technology improves the predictive validity of laboratory models. A second limitation of our study is that the current results are limited to spider-fearful female university students, and possibly cannot be generalized to male participants, groups with other ages and educational background, and spider-phobic patients. We had strong a priori reasons to focus specifically on female participants because spider phobia is much more common among women. Nonetheless, gender remains an important factor to consider in future studies because gender differences are quite pronounced in anxiety disorders (Fredrikson et al., 1996; Gater et al., 1998), which may be related to differences in fear conditioning and extinction processes between genders (Lebron-Milad et al., 2012; Milad et al., 2006). Thus, future research may investigate gender differences in fear conditioning processes in VR. A third limitation is that we did not assess participants' presence in the VR environment using a standardized questionnaire (Witmer & Singer, 1998). Variation in the amount of presence may moderate the amount of anxiety which participants' experience while being in the VR environment (Ling et al., 2014). We encourage future studies investigating fear conditioning in VR to take presence in the VR environment into account. Finally, our study focused on one specific fear-related disorder, namely spider fear and phobia. While we think that many of our arguments apply to other anxiety-related disorders as well (such as other specific phobias, social phobia, and generalized anxiety disorder), clearly more research is required to confirm that VR offers a useful tool to create valid simulations of the fear acquisition- and extinction-related processes in these disorders as well.

In conclusion, the results of our study demonstrate that cue conditioning using an ecological valid US can be conducted using VR. Our results support the evidence for VR as a promising tool to improve the validity fear conditioning research. Future studies should focus on further extending and exploiting the technological advances of VR technology to bridge the translation gap between fundamental research and treatment.

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