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A Novel Sensory Pre-Conditioning Paradigm to Assess Inferential Fear Generalization

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Laymen Summary

We all fear something, whether it is spiders, heights, sharks, or dentists. Fear benefits us as it keeps us away from potentially dangerous objects or situations. In some cases, the specific fear we have for something can also spread to other things; this process is called fear generalization. For example, a person might experience a traumatic dog attack. Later, they might also learn to fear other dogs based on shared physical characteristics, such as having four legs.

When fear generalization becomes excessive, it no longer benefits us. In fact, extreme fear generalization is the cause of many anxiety disorders. Going back to the previous scenario, a person who was attacked by a dog may be so traumatized that fear spreads to any other object, situation, or person previously associated with dogs, such as a neighbor who owns a dog. To generalize this form of fear, the person would have to infer that seeing the neighbor signals the presence of a threat nearby. Thus, it would be based on the previous associations formed in a person's mind, such as knowing that the neighbor has a dog. This type of fear generalization is called “inferential fear generalization.”

Although inferential fear generalization is adaptive, if it becomes excessive, it may negatively affect a person's daily life. Consequently, the person might show exaggerated fear responses to different circumstances, objects, or people regardless of whether they pose a threat. Particularly, people who experienced trauma in their childhood were found to be more likely to show excessive fear generalization. Thus, understanding the underlying processes behind inferential fear generalization is important for both the prevention and the treatment of these disorders.

To study inferential fear generalization, we designed a new paradigm with three phases:
1) teach participants the association between images in a sequence
2) pair the last image with an electric shock so that participants learn to fear the specific image
3) observe whether inferential fear generalization takes place to other images in the sequence

We also wanted to see if inferential fear generalization requires mental effort. Thus, we divided participants into two groups in which they completed a task that required a lot of mental work or almost none. The images we used were not similar; thus, the generalization would be based only on the association participants formed in their minds. Additionally, we wanted to see if there was a correlation between childhood trauma and fear generalization.

Even though participants learned to fear the image presented with the shock, they did not show any fear generalization to other images. In addition, the different tasks completed by the two groups did not affect the results. Finally, we did not find any evidence for the relationship between fear generalization and childhood trauma.

In summary, our work was the first to investigate inferential fear generalization using a new paradigm with sequence, allowing us to quantify how much fear is generalized to images inside the sequence. As a result, this study establishes the framework for future inferential fear generalization research to improve.

Abstract

Fear generalization is a survival mechanism that helps us predict threat and adjust our responses to potentially dangerous stimuli. Thus, through fear generalization, the fear response can transfer from the original threat predictor to other stimuli. Even though adaptive, over-generalization of fear is the hallmark of anxiety disorders; thus, studying its underlying processes is essential for both preventing and treating anxiety-related disorders. Prior research shows that fear generalization may also occur in cases where there is no direct pairing between the threat predictor and the threat itself. A high-order inference is required for this kind of generalization. A possible underlying mechanism in the human brain for this inference process is “spontaneous experience replay." This process takes time, requires cognitive resources, and as a result, the replay allows the attribution of previously learned associations to new stimuli. However, inferential generalization within the context of fear has not yet been explored. To address this gap, we developed a three-phase sensory pre-conditioning paradigm exploring inferential fear generalization. Additionally, previous research found that childhood trauma impairs fear learning. However, its relationship with inferential fear generalization has never been explored. Thus, this study aimed to 1) establish a novel sensory pre-conditioning paradigm and investigate whether we could interfere with the revaluation necessary for inferential fear generalization by manipulating cognitive load 2) examine whether there was a correlation between childhood trauma and inferential fear generalization. The results suggest that the paradigm did not lead to fear generalization. Even though there was successful fear conditioning, there was no retention of the fear during the first trial of the test phase, which may have caused the lack of generalization. Also, there was no correlation between childhood trauma and fear generalization, which could have been due to the absence of fear generalization. In summary, our study was the first to use a sensory pre-conditioning paradigm with sequences to explore inferential fear generalization, allowing us to measure how much fear generalizes to stimuli inside the sequence. It was also the first study to look at how cognitive load affected this process and if inferential fear generalization was connected to childhood trauma. Thus, this study lays the groundwork on which future inferential fear generalization studies can be improved upon.

Keywords: inferential fear generalization, sensory pre-conditioning, higher-order inference, spontaneous experience replay

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1. Introduction

1.1 Introducing Fear Generalization

Fear learning is an adaptive mechanism for survival that helps us to associate certain cues with potential threats and learn defensive responses to protect ourselves (Dymond et al., 2015). This learning needs to be flexible enough to recognize new stimuli as similar to the previously learned fear situations so that a person can anticipate and prevent harm (Dymond et al., 2015). Thus, fear learning is not limited to the exact instances in which conditioning takes place but rather includes a generalization process by which we adjust our responses to newly encountered stimuli (Dunsmoor et al., 2011). This process of transferring fear to other stimuli is called "fear generalization" (Dunsmoor et al., 2009). For example, a person that previously fell on an escalator and hurt themselves badly might later become scared of all escalators regardless of their size, location, and other external characteristics. Despite being highly adaptive and useful, fear generalization may also have negative implications (Dunsmoor et al., 2009). Indeed, the overgeneralization of fear characterizes many anxiety and post-traumatic disorders (Mertens et al., 2021). Therefore, fear generalization is considered a key component of anxiety, causing excessive reactions to a wide variety of items, people, and situations despite their diversity; accordingly, studying its underlying mechanisms is crucial for both preventing and treating these clinical disorders.

Our current understanding of fear generalization processes is fundamentally based on the classical fear conditioning paradigm described by Pavlov (Pavlov, 1927). Pavlov demonstrated that the conditioned response (CR) of animals following conditioning training is generalizable to a variety of stimuli (Pavlov, 1927). In the classical paradigm, an unconditioned stimulus (US) that elicits fear, e.g., a shock, is paired with a conditioned

stimulus (CS), e.g., an auditory tone. If the CS is a good predictor of the US, a CS-US association or fear memory is created, and the CS starts to elicit a CR that is analogous to the one evoked by the US. Based on this paradigm, many studies investigated fear generalization focusing on stimulus intensity, perceptual similarity, and conceptual similarity - all of which may affect the fear response (Dunsmoor et al., 2017; Dunsmoor & Murphy, 2015). These studies revealed that fear generalization increases together with the intensity of the stimulus, physical resemblance, and conceptual similarity of the stimuli. However, it is also possible for other cues that are not directly related to a fearful situation to evoke a fear response (Dunsmoor et al., 2011). For example, intense fear can be triggered by stimuli or situations that were perceived to be associated with predictors of a traumatic event but were themselves temporally and/or spatially separated from it. Thus, fear generalization may occur due to prior associations formed between a predictor of threat and other cues. As an example, a person who experienced a traumatic dog attack might not only learn to fear other dogs based on shared physical traits, such as having four legs, but this attack might be so traumatic that the fear spreads to elements that were previously associated with dogs, such as everyone who owns a dog, e.g., the neighbor (Dymond et al., 2015). This type of fear generalization would require the person to make an inference that seeing the neighbor means there is a threat close by. This process, referred to as inferential fear generalization, necessitates a high-order inference based on learned associations between stimuli (Ahmed & Lovibond, 2015; Dunsmoor & Murphy, 2015). Higher-order conditioning is highly relevant from a clinical perspective as excessive fear generalization in anxiety disorders is often described as arising from higher-order fear learning processes (Mineka & Zinbarg, 2006). Despite the relevance of this phenomenon for anxiety disorders, there is little to no research on this topic compared to other forms of generalization.

1.2 Underlying Mechanisms Behind Inferential Fear Learning

In contrast to first-order conditioning, higher-order conditioning or inferential learning is suggested to be mediated by the cooperation of two different systems: Model-Free (MF) and Model-Based (MB) learning (Gershman et al., 2014). MF learning comprises learning of the previously experienced CS – US pairs (e.g., dog and the attack). Thus, it operates on direct experience (e.g., the model-free system can learn that seeing a dog predicts an attack; thus, the person becomes scared of dogs). MB learning, on the other hand, operates on simulated experience (Gershman et al., 2014). Here, the previously learned CS-US pairs are replayed from memory, and connections between them are reevaluated. As a result, transitions between the CS's and the US's in the environment are made, such as updating the perceived threat from all dog owners after experiencing a dog attack. Such simulations are proposed to take place after learning via offline "spontaneous experience replay" (Gershman et al., 2014). This process is based on a retrospective revaluation of the previous associations between the natural cues and the threat predictors, which then updates the values accordingly. Thus, it is suggested to support inferential generalization (Gershman et al., 2014). In line with this, Dunsmoor and colleagues demonstrated that when retroactively paired with a US, the memory for previously neutral objects was substantially improved after fear conditioning (Dunsmoor et al., 2015). These findings support the hypothesis that a retroactive memory process selects and updates the values of stimuli that were not themselves paired with a US (Dunsmoor et al., 2015). Furthermore, these backward updates in memory were only observed after a period of temporal delay (Dunsmoor et al., 2015). In all of these studies, stimuli were, however, all conceptually related.

Research has also shown that MB learning is cognitively demanding (Gershman et al., 2014). For example, Gershman and colleagues demonstrated that the degree of retrospective

revaluation was decreased by high cognitive load (Gershman et al., 2014). In line with these results, cognitive load has been hypothesized to influence fear generalization responses (Kamphuis & Telch, 2000). It was observed that cognitive load on working memory impairs fear learning and, as a result, leads to elevated fear generalization responses (Manbeck et al., 2022).

Thus, the aim of our study was twofold:

1) Whether spontaneous replay would also mediate the generalization of fear to stimuli that are not conceptually related to the CS but merely associated with it by prior learning – as in inferential fear generalization.

2) Check whether we can interfere with this process by increasing cognitive load.

1.3 A Sensory Pre-Conditioning Paradigm to Study Inferential Fear Generalization

To study classical fear generalization in a laboratory setting, experiments commonly employ a paradigm whereby one stimulus (CS+) is paired with an unpleasant outcome, such as an electric shock, whereas the other one (CS-) serves as the safety cue - it is never paired with a shock (Lis et al., 2019). Following this, fear responses to conceptually related stimuli are measured to assess whether fear responses generalize to them. However, compared to these experiments, there is very little human research on inferential fear generalization (Dunsmoor et al., 2011). Therefore, the characteristics that encourage fear to generalize across previously associated stimuli which are not conceptually related are unexplored. To investigate inferential fear generalization, a more complex paradigm called “Sensory Pre-Conditioning” can be employed (SPC; Dunsmoor et al., 2011). This paradigm makes it possible to measure fear generalization responses based on the prior associations formed

between stimuli (Dunsmoor et al., 2011). The conventional SPC paradigm is divided into three phases (Dunsmoor et al., 2011). During the first phase, i.e., the pre-conditioning phase, participants learn to associate two neutral cues, S1 and S2, e.g., a tone and a light. In the second phase, one of the cues, i.e., S2, is paired with a US, such as an electric shock, which results in a CR. In the third phase, only the S1 is presented, which has never been paired with the US. Due to the association formed between the S1 and S2, the presentation of S1 also leads to a CR. In short, the pairing of S1 and S2 occurs before S2 is paired with the US. In contrast to the conventional SPC, where two stimuli (i.e., S1 and S2) are used, our paradigm introduces a sequence of stimuli that allow us to investigate the degree to which fear generalizes to each stimulus.

1.4 Inferential Fear Generalization and its Relationship with Childhood Trauma

While inferential fear generalization is adaptive, if it becomes extreme, it may affect people's social functioning and interfere with everyday life (Mertens et al., 2021). For example, people with post-traumatic disorders show elevated fear responses to stimuli and circumstances that are not only harmful or conceptually match the context in which the initial trauma happened but also to those stimuli and circumstances that are safe or merely associated with the predictors of the traumatic event (Mertens et al., 2021). Thus, experiencing a traumatic event may lead to maladaptive inferential fear generalization patterns.

The research found that fear learning is impaired in people who have experienced childhood trauma (Lis et al., 2019). These people showed higher fear responses to safety signals and as a result, failed to differentiate between the safety and the danger cues (Lis et al., 2019). However, the relationship between childhood trauma and inferential fear

generalization has not yet been explored. We hypothesized that fear generalization responses would be higher in people with childhood trauma than the rest of the population, as experiencing childhood trauma leads to inaccurate identification of safety signals. As a result, we would anticipate those who have experienced childhood trauma to misinterpret safety signals as dangerous and also show fear responses to them. Based on this assumption, the third aim of this study was to explore the degree to which people generalize fear was correlated with childhood trauma. We used the Childhood Trauma Questionnaire to assess childhood trauma (CTQ; Bernstein et al., 1994).

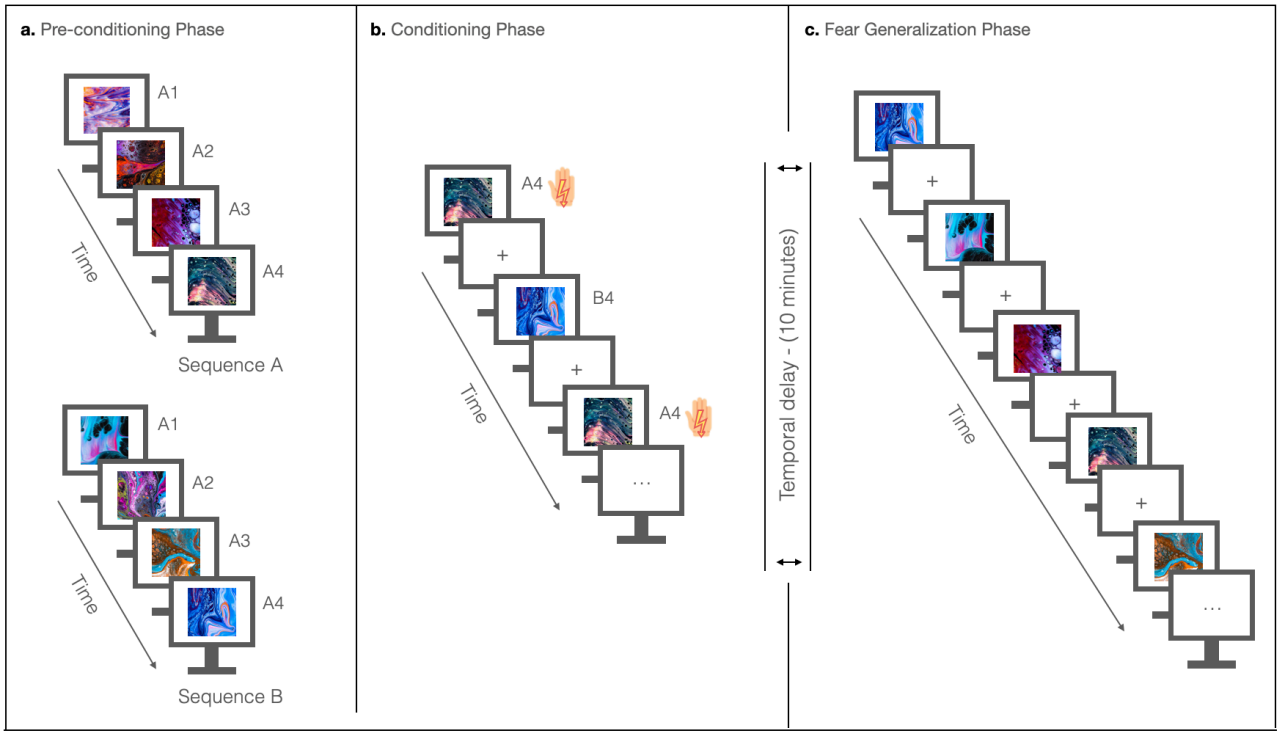
1.5 Experimental Design

As mentioned above, we designed a new sensory pre-conditioning paradigm with three phases: pre-conditioning, conditioning, and fear generalization ,(Figure 1) to study whether inferential fear generalization requires a time-dependent spontaneous replay of the events and whether cognitive load interferes with this process.

In the pre-conditioning phase, participants were presented with two sequences (A and B) of 4 fractal pictures each: CSA1, CS2, CS3, CS4, and CSB1, CSB2, CSB3, and CSB4. This phase aimed to form associations between stimuli for each sequence. Thus, participants were instructed to learn the order in which the fractals are presented. Fractals were presented in a fixed order within the sequences for all participants. In the fear conditioning phase, the last item in Sequence A (i.e., CSA4+) was paired with an electric shock, while the last item in Sequence B (i.e., CSB4-) was the control stimulus, i.e., it was not paired with the electric shock. Thus, Sequence A served as the reinforced sequence and Sequence B as the unreinforced sequence. CSA4+ was paired with an electric stimulus with a 75% reinforcement rate (defined as “maximum uncomfortable but not painful”). During this phase,

Figure 1

Experimental Design of the Sensory Pre-Conditioning Paradigm



Note. This figure demonstrates the experiment distributed over three phases. **a.** Pre-conditioning phase: participants learn neutral stimuli sequences. **b.** Conditioning phase: the last stimulus of each sequence is paired with one of the two: an electric shock or left unpaired. After conditioning phase, participants are separated into two groups: a 10-minute delay group with low (0-back) or a high (2-back) cognitive load manipulation. **c.** Fear generalization phase: generalization of the fear is assessed with stimuli from phase 1.

participants were asked to pay attention to the images on the screen. In the fear generalization test phase, participants were randomly assigned to two cognitive load conditions: low or high cognitive load - both of which lasted 10 minutes. The n-back task was chosen to manipulate the cognitive load (Mehler et al., 2011). Participants in the low cognitive load group completed a 0-back task, and participants in the high cognitive load group completed a 2-back task. After the cognitive load task, participants received the instructions that the experiment would continue. Both groups were shown fractal images from phase 1 in a

pseudo-randomized way (e.g., CSA4/B4, CSA3/B3, etc.). This design enabled us to measure how fear generalization responses differ based on the order of each stimulus in the sequence. The primary outcome measures were Fear Potentiated Startle Response (FPS), as it is one of the most common measures employed in fear generalization studies (Ahmed & Lovibond, 2015; Dunsmoor & Paz, 2015; Dymond et al., 2015). We additionally collected Skin Conductance Response (SCR) which was not the focus of the present thesis. Finally, for sample characterization, we measured anxiety disorder through Spielberger State-Trait-Anxiety Inventory (STAI; Spielberger et al., 1983), anxiety apprehension through the Anxiety Sensitivity Index (ASI-3; Taylor et al., 2007), and worry about uncertain situations with the Intolerance of Uncertainty scale (IUS; Freeston, Rheaume, Letarte, Dugas & Ladouceur, 1994).

This study explored the following hypotheses:

1. If inferential fear generalization is based on a replay process, then the stimuli preceding the CSA4+ but not CSB4- should also evoke a generalized fear response after a temporal delay. Thus, we can expect a gradient of fear responses by the stimuli preceding the CSA4+ (i.e., CSA3>CSA2>CSA1).
2. If cognitive load interferes with this inferential process, fear generalization should only be observed in the low cognitive load condition.
3. If childhood trauma impairs fear generalization, then we would expect a correlation between the generalization index (i.e., calculated as the difference between the averaged fear responses to CSA1-3 and CSB1-3) and the CTQ scores. Specifically, we expect a positive correlation between the CTQ scores and the degree of fear generalization.

2. Methods

2.1 Participants

Our sample size estimation was based on a previous pre-conditioning study in which conceptually unrelated stimuli were used to test fear generalization (Dunsmoor et al., 2011). The results of this study were adopted to calculate the effect size, r = 0.53, based on a paired t-test, t (28) = 2.87, $p < 0.01$. The required sample size was N = 30 (two-tailed paired t-test), calculated using the effect size 0.53, a significance level of 5% to reach a power of 80%. Thus, by recruiting 30 participants per group (60 participants in total), it would be possible to detect fear generalization responses to the first pre-conditioned stimuli using a rmANOVA with eight repeated measures (2 sequences with 4 CSs each) with a small effect size of f = 0.125. The final sample included 41 participants, of which 31 were females and 10 were males. Due to the time limitations of the present thesis, the initial sample size calculation was not met. Participants were recruited by the SONA platform of Utrecht University, the lab website (lab.uva.nl) of the University of Amsterdam, and advertisements around the Utrecht Science Park campus. Participants who signed up for the study via SONA could receive 1.5 PPU credits, and those from the lab website could either receive 15 research credits or 15 euros. In order to be eligible for the study, participants were required not to have color blindness and have a good level of English language. In preparation for the study, all participants were randomly assigned to one of the cognitive load conditions. Participants (N=30/8 female/male) were between 17 and 44 years old (M=21.5, SD=4.8). When asked about their current occupation status, all of them said, “student." EMG data of three participants had to be disregarded for further analysis, one due to not completing the second phase and the other two due to not passing the quality check of the electromyography

recordings, leaving a final sample size of N = 38. The study was approved by the Ethics Committee of the Faculty of Social and Behavioral Sciences of Utrecht University and by the Ethical Review Board of the University of Amsterdam. The study was conducted in accordance with the Declaration of Helsinki.

2.2 Stimuli

2.2.1 Unconditioned Stimulus (US)

The delivery of an electric stimulus to the left wrist of the participant served as the unconditioned stimulus. The delivery was through 2 x 20 by 25 mm Ag/AgACl electrodes and fixed inter-electrodes mid-distances of 45 mm. A conductive gel was used to fill the stimulus electrodes (Signa Gel, Parker Laboratories Inc.). The US delivery was made up of a repetition of three square-wave pulses with 2 ms each and 100 and 200 ms between each pulse. The US delivery was controlled via the Digitimer DS7A constant current stimulator (Digitimer Ltd., Hertfordshire, UK). The calibration of US intensity was done individually for each participant according to their subjective ratings until a rating of 9 out of 10 was achieved on the rating scale (i.e., 0= I do not feel anything, 5= medium uncomfortable shock, 9= maximum uncomfortable but tolerable, 10= already painful shock). Participants could change the intensity, stop or withdraw from the experiment at any time. Subjective intensity ratings had a mean±s.d. of 8.9 ± 0.24 (ranging from 7.5 to 9). Objective US intensity ranged from 4 to 95 mA with a mean±s.d. of 29.10±18.75 mA.

2.2.2 Conditioned Stimuli (CS)

Sixteen fractal images served as CSs. Fractals were chosen to avoid any previous associations and emotional valence with real-life objects. All of the fractal images were found on open-source websites. By randomizing the assignment of fractal pictures to CSA1-4 and CSB1-4, two sequences (A and B) of 4 fractals were created with different images. Subsequently, the order was fixed, and all the participants were presented with the same sequences. The remaining eight images served as control stimuli to test whether participants could identify the images used during the experiment in a recognition memory test after the experiment. This test was used to ensure that participants explicitly recall the fractal images used in the experiment so that a lack of recognition does not prevent fear conditioning.

2.3 Measurements

2.3.1 Childhood Trauma Questionnaire (CTQ)

A version of the Childhood Trauma Questionnaire was used to test the correlation between inferential fear generalization and childhood trauma (CTQ; Bernstein et al., 1994). Due to the total time limitations of the study, we collected data for the short form of the Childhood Trauma Questionnaire (CTQ-SF). CTQ -SF included 28 items to assess childhood trauma on a 5-point Likert scale from 1 (never true) to 5 (very often true).

2.3.2 Fear Potentiated Startle Response (FPS)

Electromyographic activity (EMG) was acquired through two 7mm Ag/AgCl electrodes attached under the eye approximately two centimeters apart. The first electrode was placed right under the pupil, and the second one towards the outer corner of the eye,

below the lateral canthus. Both electrodes were filled with conductive gel. Additionally, two electrodes were attached to the forehead at an electrically neutral location to serve as ground electrodes. In order to prompt the startle reflex, a loud noise (40 ms; 104dB) was presented binaurally through the headphones (Model Senneisser) in all of the trials. BioSemi ActiveTwo system (BioSemi Instrumentation, Amsterdam, The Netherlands) was used to record and amplify the EMG signal. Subsequently, offline analysis of the signal was done with Psycho-Physiological Modeling (PsPM 5.0.035 in Matlab 2020a, Mathworks ®, Natrick, Massachusetts, USA) in Matlab. After band-pass filter (cut-off: 50Hz and 470 Hz, 4th order Butterworth filter), a notch filter was applied to the signal to remove the 50 Hz noise. Moreover, a 4th-order Butterworth low-pass filter with a temporal constant of 3 ms was used to correct and smooth the signal. Data was then down-sampled to 500 Hz. Visual quality control was performed on the resulting pre-processed EMG data. To estimate trial-by-trial Fear Potentiated Startle (FPS) responses, we implemented a single-trial general linear model (GLM). The GLM employed a convolution operation using one individual regressor for each startle-probe onset and a standard startle response function with variable response onset delay between 0-100ms. Before the statistical analysis, single-trial parameter estimates were Z-transformed for all the participants individually between stimuli (excluding habituation trials) and phases.

2.3.3 Skin Conductance Responses (SCR)

Electrodermal activity (EDA) was measured through two AG/AgCl Electrodes (20 mm x 16 mm) from the index and middle fingers of the left hand. The resulting EDA was recorded with the BioSemi ActiveTwo system (BioSemi Instrumentation, Amsterdam, The Netherlands). SCR was not analyzed for the present thesis.

2.3.4 Questionnaires

For sample characterization, we used the Spielberger State-Trait-Anxiety Inventory (STAI; Spielberger et al., 1983), the Anxiety Sensitivity Index (ASI-3; Taylor et al., 2007), and the Intolerance of Uncertainty scale (IUS; Freeston, Rheaume, Letarte, Dugas & Ladouceur, 1994). The STAI consists of two parts, Y-1 and Y-2, both of which include 20 items. Items 1–20 assess situational/state anxiety (STAI-S), i.e., how an individual feels at the moment, whereas items 21–40 assess trait anxiety (STAI-T), i.e., how participants feel in general. The responses were given on a Likert scale ranging from “not at all” to “very much so." The ASI comprises 18 items, measuring the degree to which the anxiety-related symptoms are distressing to the person, possible responses being 0=very little, 1=a little, 2=some, 3=much, 4=very much. Finally, IUS was used to explore excessive worry about ambiguous or uncertain events and their behavioral, cognitive, and emotional effects on the individual with 27 items on a scale ranging from 1 (Not at all characteristic of me) to 5 (Entirely characteristic of me).

2.4 Experimental Procedure

The experiment took place in the Experimental Psychology facilities of Utrecht Science Park and the Behavioral Science facilities of the University of Amsterdam. Two experimenters collected data for the study. Experimenter 1 collected data at Utrecht University, and experimenter 2 collected data at the University of Amsterdam. Both experimenters followed the same experimental procedure. When participants arrived at the lab, they were provided an introduction letter outlining the general procedure of the study. After that, they were asked to fill in and sign the informed consent form. Consequently, the

participants were asked demographic questions and filled out the STAI-S Form. When the survey was completed, the experimenter attached the electrodes and began the study.

2.4.1 Phase 1: Pre-Conditioning

After the calibration and an additional check of the recordings, the participants put on the headphones, which they wore for the duration of the experiment. Shortly before the beginning of the pre-conditioning phase, participants were told that they would view various images on the screen and were instructed to memorize the sequence in which they would appear. They were also told that during the course of the trial, loud noises might be heard via the headphones, with a constant white noise present in the background throughout the experiment. 10 Noise alone (NA) trials were presented before starting the actual pre-conditioning phase for startle response habituation. Following this, the CSA1-CSA4 and CSB1-CSB4 sequences were presented three times each, interspersed with 6 NA trials. The trial orders of the sequences were randomized in a way that no more than two trials of the same type (i.e., sequence A, B, NA) were presented after each other.

During the pre-conditioning phase, fractal pictures were presented for 3.5 seconds in the middle of the screen. A startle probe was presented 3 seconds after the CS onset. The last item in sequence A was used as the conditioned stimulus (CS+), and the last item in sequence B as the neutral stimulus (CS-). Inter trial intervals (ITI) were randomized to be between 15-18 seconds, during which a fixation cross appeared in front of a black background. Based on the results of a pilot study conducted prior to the experiment, it was observed that participants had difficulties learning the order of the stimuli in each sequence. Accordingly, Phase 1 was repeated three times in this experiment to ensure that participants learned the

correct order, i.e., Phase1A, Phase1B, and Phase1C. After each phase 1, there was a memory test to assess the order of the stimuli.

2.4.2 Order Memory Test 1

To exclude that a lack of order learning causes a lack of fear generalization, we tested whether participants learned the correct order of stimuli in each sequence (CSA and CSB) following pre-conditioning. During this order memory test, participants were presented with all CSA and CSB stimuli and instructed to indicate whether the stimulus was presented (1) first, (2) second, (3) third, or (4) fourth/last in its sequence.

2.4.3 Phase 2: Fear Conditioning

The final CS of each sequence (CSA4, CSB4) and the NA trials were randomized and delivered eight times each during the fear conditioning phase. 75% of presentations used electric stimulation to reinforce the CSA4+, while the CSB4- was never followed by a shock. The randomization of trial order ensured that no two consecutive presentations of identical stimulus occurred. Every CS was shown for 6.5s and 6s after the onset of the stimulus a startle probe was presented. When trials were reinforced, the US ended with the CS offset. Between the trials, an ITI of 15–18s was used.

2.4.4 Cognitive Load Manipulation

Following the fear conditioning phase, there was a 10-minute pause. During this delay, participants were divided into one of the two cognitive load conditions, i.e., low or high cognitive load. Participants in the low cognitive load group did a 0-back task, while participants in the high cognitive load group did a 2-back task.

2.4.5 Fear Generalization Test

After the cognitive load manipulation, participants were instructed that the experiment simply continues. During the generalization test, CSs (CSA1-CSA4 and CSB1-CSB4) were presented twice in a pseudo-randomized order throughout the test phase. Additionally, there were 8 NA trials. To avoid new learning, the generalization test was conducted in extinction, i.e., none of the CSs were combined with an electric shock. Presentation, startle probe onset, and ITI occurred simultaneously during the previous conditioning phase. The order of the trials was randomized so that no more than two trials of the same stimulus were presented after each other.

2.4.6 Recognition Memory Test and Order Memory Test 2

Subsequently, participants took an object recognition test and an order memory test. To begin, they were asked to press O or N on the keyboard to indicate whether a fractal image displayed on the screen had previously been seen during the experiment (old) or had never been seen before (new). Participants were also asked to rate their certainty on a scale of 0 (not at all certain) to 3 (completely certain). Second, we used an order memory test to see if people remembered the order in which the CSs were presented to exclude the possibility that a lack of remembering the correct order causes a lack of fear generalization. The format was the same as the phase 1 test. Finally, to test the CS-US contingency awareness, participants were asked to indicate whether the CSA4+ or the CSB4- was paired with an electric stimulus.

2.4.7 Similarity Test

In addition, participants completed a similarity test. During the test, two fractal images used in the experiment appeared on the screen. Participants were instructed to give a

rating to the images ranging from 0 to 100 (i.e., 0=not similar to 100=extremely similar) based on how similar they were. The results of this test are not the focus of the current thesis.

2.4.8 Questionnaires

Following the removal of the headphones and electrodes, the participants filled out the STAI-T, the ASI-3, the CTQ-SF, and the IUS. Finally, participants were informed about the study's purpose.

2.5 Statistical Analysis

All statistical analyses were performed using the R statistical software (version 4.1.2). All data were examined by repeated measures ANOVA tests with an alpha level of $p < 0.05$ (two-tailed). Prior to statistical analysis of the conditioning data, the FPS responses for CSA4+ and CSB4+ were averaged across the first two and last two trials (out of 8 trials in total) for the conditioning phase. To evaluate whether fear conditioning was successful, as it is necessary for generalization to take place, a repeated measure ANOVA (rmANOVA) was conducted with the stimulus (CSA4+, CSB4) and trial (first two and last two trials) as within-subject and group (low or high cognitive load) and experimenter (experimenter 1, experimenter 2) as between-subject factors of FPS responses from the conditioning phase. To check whether conditioned fear was successfully retrieved after the cognitive load manipulation in the generalization phase, a rmANOVA with group (low or high cognitive load) as between-subject and stimulus (CSA4+ and CSB4-) as within-subject was run using the first trial FPS responses. To test whether fear generalized from CSA4+ to other stimuli in Sequence A, a rmANOVA was performed with sequence (reinforced sequence A vs. unreinforced sequence B) and order (i.e., CSA1, CSA2, CSA3 and CSB1, CSB2, CSB3) as

within-subject, and group (low or high cognitive load) and experimenter (1 or 2) as between-subject factors on the first trial. Finally, to explore the correlation between CTQ scores and fear generalization, Spearman's correlation coefficient was calculated. In order to obtain a generalization score for each participant, the difference between the averaged FPS responses to CSA1-3 and CSB1-3 was used. The various tests' presumptions were examined, and any violations were considered when interpreting the results. Results were considered statistically significant when $p < 0.05$.

3. Results

3.1 Order Memory Test 1

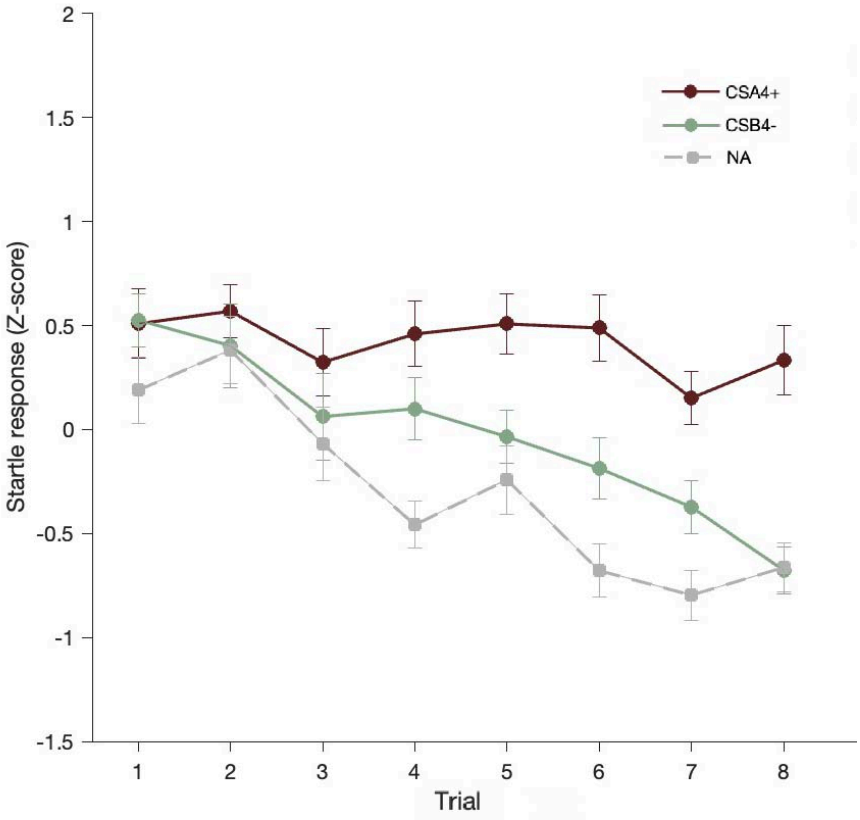
To ensure that participants learned the correct order, test data was collected three times in total (i.e., Order Memory Test 1A, 1B, and 1C) for all participants after each of the phase 1 sessions. Participants scored on average 7.88 ± 0.47 s.d. out of 8 (answers ranged from 6 to 8). After the third practice round (i.e., Phase 1C), 36 participants out of 38 acquired the correct order of the fractal images in each sequence (i.e., Sequence A and Sequence B). In comparison, the remaining two participants still made some mistakes in their responses to Test 1C.

3.2 Fear Conditioning

To test whether the fear conditioning was successful (i.e., whether participants showed elevated fear responses to CSA4+ and not to CSB4-), we looked at the FPS response amplitudes. Figure 2 depicts FPS responses throughout the trials of the fear conditioning phase to CSA4+ and CSB4-. A rmANOVA confirmed these findings for the stimulus

Figure 2

Fear Conditioning Phase



Note. This graph depicts the startle responses during the conditioning phase over eight trials for the CSA4+, CSB4-, and NA. Error bars display the standard error of the mean.

(CSA4+, CSB4-) and trial (first two and last two trials) within-subject variables and the group (low or high cognitive load) and experimenter (1 or 2) as between-subject factors. There was a significant effect of stimulus F (1,34) = 13.78, p = 0.0007, η_G^2= 0.07 and trial F (1,34) = 12.91, p = 0.001, η_G^2= 0.09 with a significant two-way interaction between the stimulus and the trial F (1,34) = 7.04, p = 0.01, η_G^2= 0.05. Neither the group of participants nor the experimenter did have any significant effect (group: F (1,34) = 2.09, p = 0.16, η_G^2= 0.02; experimenter: F (1,34) = 0.19, p = 0.66, η_G^2= 0.002; group x trial: F (1,34) = 0.01, p = 0.94, η_G^2= 0.00004, group x stimulus: F (1,34) = 0.73, p = 0.40, η_G^2=0.004; group x

experimenter: F (1,34) = 0.83, p = 0.37, η_G^2= 0.007; experimenter x trial: F (1,34) = 0.69, p = 0.41, η_G^2= 0.005; experimenter x stimulus: F (1,34) = 0.01, p = 0.94, η_G^2= 0.00003). To further explore the interaction effect between stimulus and trial, we ran a one-way model of the stimulus (CSA4+ and CSB4-) at each level of the trial (first two and last two). The results with the Bonferroni adjusted p-value demonstrated that the simple main effect of the stimulus was not significant in the first two trials, p = 0.65, and in the last two trials, p = 0.00001, suggesting that fear conditioning to the CSA4+ vs. CSB4- was successful at the end of the conditioning phase. Thus, the combined findings imply that fear training was successful regardless of the group and the experimenter.

3.3 Fear Generalization Test

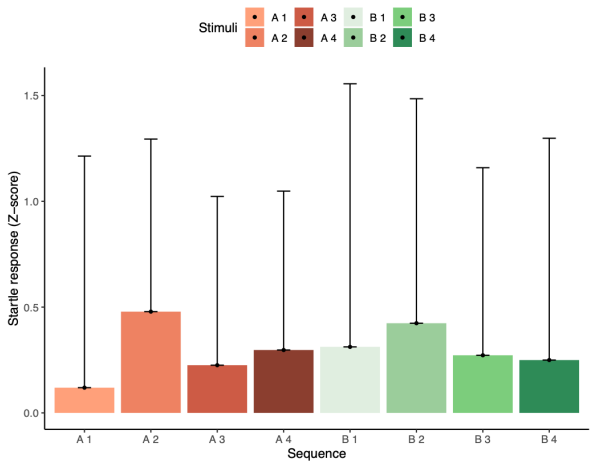
To test whether there was any fear retrieval after the cognitive load manipulation, we first conducted a rmANOVA with group (low or high cognitive load) as between-subject factor and stimulus (CSA4+ and CSB4-) as within-subject factor as measured by the FPS responses on the first trial of the generalization test phase. Even though there was a mean difference of 0.08 between FPS to CSA4+ (M=0.30, SD= 0.78) and CSB4- (M=0.22, SD= 0.91), the difference was not significant F (1,36) = 0.27, p = 0.061, η_G^2= 0.002 suggesting that there was no fear retention from the conditioning phase. Furthermore, the group of the participants did not have any significant effect on fear retention F (1,36) = 0.01, p = 0.91, η_G^2= 0.0002, and there was no interaction between stimulus and group F (1,36) = 0.13, p = 0.72, η_G^2= 0.001. These results indicate no fear retention regardless of the cognitive load condition. This may be due to short-term conditioning effects produced by the fear conditioning phase.

As planned before, rmANOVA of FPS responses from the first trial of the generalization phase were used to evaluate the generalization of the fear with sequence (Sequence A vs. B) and order (CSA 1-3 and CSB1-3) as within-subject factors and group (low or high cognitive load) and experimenter (1 or 2) as between-subject factors (Figure 3). In contrast to our hypotheses, there was, however, no significant main effect for sequence F (1,34) = 0.81, p = 0.78, η_G^2=0.0003 or order F (2,68) = 0.27, p = 0.76, η_G^2 = 0.003 and no significant interaction between sequence and order (F (2,68) = 0.76, p = 0.47, η_G^2 = 0.006), suggesting that fear did not generalize from CSA4+ to CSA1-3 and that generalization was not affected by which sequence the stimuli were in. Furthermore, the main effect of group (F (1,34) = 0.07, p = 0.80, η_G^2 = 0.0005), as well as the interactions between group x sequence (F (1,34) = 0.16, p = 0.69, η_G^2 = 0.0006), group x order (F (2,68) = 0.60, p = 0.55, η_G^2 = 0.006) and group x order x sequence (F (2,68) = 0.66, p = 0.52, η_G^2 = 0.005) were not significant suggesting that cognitive load did not have an impact on the fear generalization. Lastly, there was no significant main effect of the experimenter (F (1,34) = 0.94, p = 0.34, η_G^2 = 0.007) and no interaction effect between the experimenter and order (F (2,68) = 1.44, p = 0.25, η_G^2 = 0.015), experimenter and sequence (F (1,34) = 1.67, p = 0.21, η_G^2 = 0.006), and experimenter and group (F (1,34) = 0.34, p = 0.57, η_G^2 = 0.003) which suggests that experimenter did not affect the generalization process. Together, these results demonstrate that fear may not have generalized as anticipated previously.

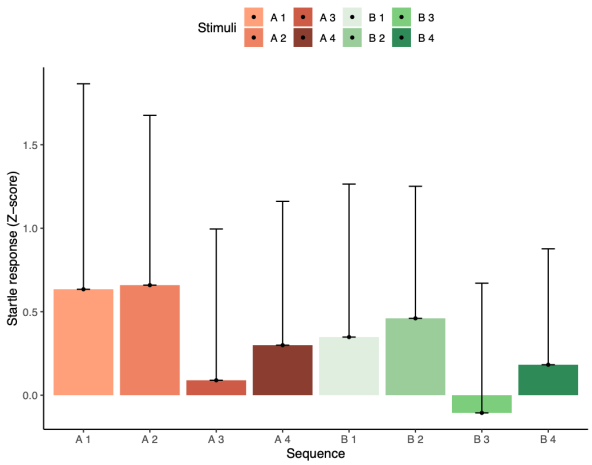
Figure 3

Fear Generalization Phase

a) Low Cognitive Load



b) High Cognitive Load



Note. The bar charts reflect the startle responses on the first trial of the generalization test phase to all of the stimuli (CSA1-4 and CSB1-4) clustered based on groups, i.e. a) low cognitive load and b) high cognitive load, with error bars depicting the standard error of the mean.

3.4 Recognition Memory Test and Order Memory Test 2

Data for both tests were collected for all participants (N=38). The results of the recognition memory test showed that 36 participants out of 38 successfully recognized the images they had previously seen during the experiment with a mean±s.d. of 15.87 ± 0.52 out of 16 (scores ranged between 13-16). These results suggest that the lack of fear generalization was not due to the inability to identify stimuli used in the experiment correctly. Answers to order memory test 2 revealed that 34 participants out of 38 still remembered the correct order of the images in both sequences with a mean±s.d. of 7.72 ± 0.86 out of 8 (scores ranged between 4-8), indicating that the lack of fear generalization was not due to the absence of memory recollection of the correct order. Finally, shock contingency ratings indicated that 32 out of 38 participants correctly identified the CSA4+ and CSB4- with a mean±s.d. of 1.82 ±

0.39, which suggests that the absence of fear generalization was not due to the explicit awareness of the shock-stimulus pairings.

3.5. Control Analysis for Fear Generalization

3.5.1 Effects of Explicit Learning of the Correct Order

Despite the good recollection of the exact order of stimuli by participants, we aimed to explore whether explicit learning would make a difference in fear generalization results. Based on this, we performed a rmANOVA for fear generalization on the FPS responses with sequence (CSA+ and CSB-) and order (CSA/B1-3) as within-subject, and experimenter (1 or 2) and group (low or high cognitive load) as between-subject factor, exclusively using data from the participants who accurately recalled the correct order in both sequences. Accordingly, these participants were determined based on their scores on order memory test 1. 36 out of 38 participants answered all the questions correctly (8 out of 8) and were included in the analysis. Nevertheless, there was no main effect of the sequence (F (1,32) = 0.10, p = 0.76, η_G^2 = 0.0004) or order (F (2,64) = 0.33, p = 0.72, η_G^2 = 0.004), and no interaction effect between sequence and order (F (2,64) = 0.60, p = 0.55, η_G^2 = 0.005) indicating that the fear generalization was not affected by explicit learning of the correct order. Moreover, neither the group nor the experimenter had any significant effect on the results (group: F (1,32) = 0.57, p = 0.81, η_G^2 = 0.0004; experimenter: F (1,32) = 0.85, p = 0.37, η_G^2 = 0.007; group x sequence: F (1,32) = 0.15, p = 0.70, η_G^2 = 0.0006; group x order: F (2,64) = 0.68, p = 0.51, η_G^2 = 0.008; experimenter x order: F (2,64) = 1.36, p = 0.26, η_G^2 = 0.015; experimenter x sequence: F (1,32) = 1.54, p = 0.22, η_G^2 = 0.006; experimenter x

group F (1,32) = 0.23, p = 0.63, η_G^2 = 0.002). These results suggest that the generalization of fear was not influenced by the explicit learning of the order of stimuli.

3.5.2 Effects of Shock Contingency Awareness

Despite the overall high accuracy in determining shock-stimulus pairings, we wanted to check if explicit awareness had an effect on fear generalization. Subsequently, we performed a rmANOVA for fear generalization only using data from the participants who correctly identified shock-stimulus pairings based on the FPS responses as outcome measures. The sequence (CSA+ and CSB-) and order (CSA/B1-3) were within-subject, and the experimenter (1 or 2) and group (low or high cognitive load) were between-subject factors. Participants were selected based on their answers to the last two questions on order memory test 2. 32 out of 38 participants correctly identified which stimulus was followed by a shock. These participants were included in the analysis of rmANOVA. However, there was no significant effect of order (F (2,56) = 0.13, p = 0.88, η_G^2 = 0.002) or sequence (F (1,28) = 0.21, p = 0.65, η_G^2 = 0.001), and there was no interaction effect between sequence and order (F (2,56) = 0.67, p = 0.52, η_G^2 = 0.006) suggesting that the fear generalization was not affected by explicit awareness of shock-stimulus pairings. Furthermore, neither the experimenter nor the group had any significant influence on the results (group: F (1,28) = 0.14, p = 0.72, η_G^2 = 0.001; experimenter: F (1,28) = 0.04, p = 0.84, η_G^2 = 0.0004; group x sequence: F (1,28) = 0.46, p = 0.5, η_G^2 = 0.002; group x order: F (2,56) = 0.49, p = 0.62, η_G^2 = 0.006; experimenter x order: F (2,56) = 1.46, p = 0.24, η_G^2 = 0.02; experimenter x sequence: F (1,28) = 1.91, p = 0.18, η_G^2 = 0.009; experimenter x group F (1,28) = 0.01, p =

0.93, η_G^2 = 0.00007). Together, these results indicate that explicit shock contingency awareness did not affect fear generalization.

3.6 Childhood Trauma

CTQ scores ranged from 34 to 73 with a mean±s.d. of 45.1±8.5. Participants' mean score was above the mean score of 30 in the non-clinical community samples (Scher et al., 2001). The mean scores for low and high cognitive groups are displayed in Table 1. To check whether the means of the two cognitive load groups differ, a t-test was used. The results demonstrated that the mean of the low cognitive load group was higher than the high cognitive load group, t (37) = 2.28, p = 0.03, d = 0.67. This significant difference was due to two outliers in the low cognitive load group. Thus, the two outliers were excluded from the calculations for further analyses.

Table 1

Mean, SD, and T-test results of CTQ scores for two cognitive load conditions

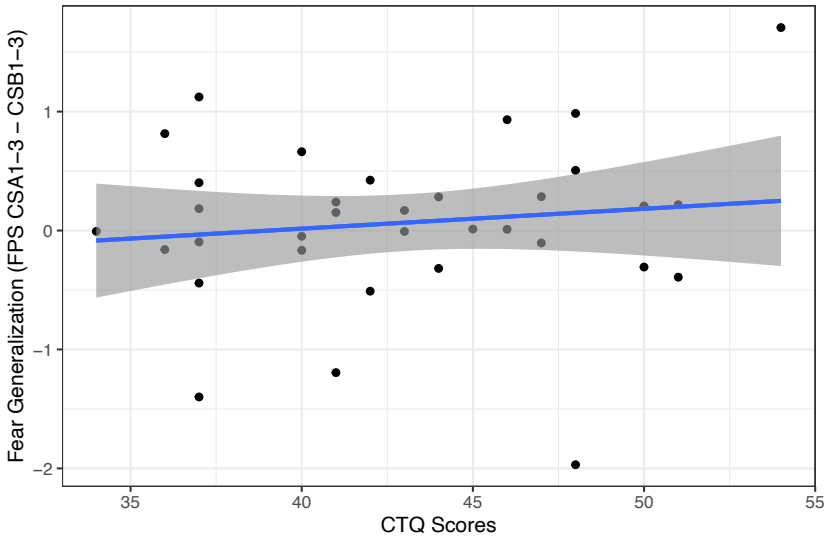
	Low cognitive load	High cognitive load	Total
Scores (Mean / s.d.)	47.4 / 10.1	41.9 / 4.0	45.1 / 8.5
T-test (t-value, p-value)			t = 2.28 , p = 0.03

To visualize the CTQ scores with fear generalization index for each participant, we first created a scatter plot using the total score on the CTQ, and the difference between the averaged FPS responses to CSA1-3 and CSB1-3 on the first trial of the generalization phase

(Figure 4). There was no correlation between the CTQ scores and the fear generalization based on the visual inspection. Additionally, we ran a correlation analysis to explore the statistical relationship between the two variables. First, the Shapiro-Wilk test was used to check for normality. As normality was not met for both variables, we ran a Spearman rank-based correlation test. The correlation coefficient between the CTQ and the fear generalization was not significant *(r* = 0.12, *p* = 0.51, Spearman). These results suggest that there was no correlation between the inferential fear generalization and childhood trauma, as previously hypothesized.

Figure 4

Correlation between fear generalization and CTQ scores



Note. Scatter plot displaying the total score on the CTQ questionnaire and the fear generalization index calculated by subtracting the averaged FPS responses to CSA1-3 and CSB1-3 for each participant on the first trial of the generalization phase.

3.7 Questionnaires

Data from each participant were collected for the STAI, ASI-3, and IUS questionnaires. The mean scores, standard deviation, and range of all the scores can be found in table 2. The results of STAI-S and STAI-T demonstrated a mean score of 31.8 (SD=6.2) and 41.8 (SD=10), respectively. STAI scores are generally divided into three categories: "no or low anxiety" (20-39), "moderate anxiety" (40-59), and "severe anxiety" (60-80) (Spielberger et al., 1983). Based on the normative values, our sample was characterized as having low state anxiety and moderate trait anxiety. The mean ASI-3 was found to be 18.3 (SD=9.8). ASI-3 scores can fall into four categories: high (54 – 72), moderate (36-53), low (18-35), and almost no anxiety sensitivity (0 - 17) (Taylor et al., 2007). Accordingly, the mean of our sample fell into the low anxiety sensitivity category. Finally, the IUS mean score was 62.4 (SD=17.5), similar to the mean score found within non-clinical samples (Khawaja & Yu, 2010).

Table 2

Mean and SD of questionnaire results

				Scores (Mean / s.d.)			
	Gender (Total)		**Age**	**STAI-S**	**STAI-T**	**ASI-3**	**IUS**
Low cognitive load	M (5)	F(17)	22.0 / 5.6	30.8 / 5.6	39.3 / 6.8	16.5 / 8.1	57.8 / 15.5
High cognitive load	M (3)	F (13)	20.9 / 3.5	33.2 / 7.0	45.2 / 12.5	20.8 / 11.5	68.6 / 18.8
Total	M (8)	F (30)	21.5 / 4.8	31.8 / 6.2	41.8 / 10.0	18.3 / 9.8	62.4 / 17.5

Subsequently, we ran t-tests to compare the mean scores between the two cognitive load groups to ensure that the sample characterization did not vary by group (table 3). The results

of the t-tests confirmed that the mean scores of the participants in both cognitive load groups were not significantly different from each other (STAI-S: t (37) = -1.12, p = 0.27; STAI-T: t (37) = -1.70, p = 0.10; ASI-3: t (37) = -1.30, p = 0.21; IUS: t (37) = -1.89, p = 0.07).

Table 3

T-test comparing the mean questionnaire scores between the cognitive load groups

	T-test results (t-value, p-value)			
	STAI-S	**STAI-T**	**ASI-3**	**IUS**
Cognitive Load	t =-1.12 , p = 0.27	t =-1.70 , p = 0.10	t =-1.30 , p = 0.21	t =-1.89 , p = 0.07

4. Discussion

This study aimed to design a novel pre-conditioning paradigm to test inferential fear generalization and investigate whether we can disrupt this process with cognitive load. Our first hypothesis was that if inferential fear generalization is based on a replay, the stimuli preceding the CS+ should evoke a fear response after a delay period. However, our findings from the FPS responses did not support the hypothesis: no fear generalization was seen in the sample. Second, given that this process requires the use of cognitive resources, we reasoned that if we interrupt this process, this would prevent the fear from generalizing. Nevertheless, fear generalization was absent regardless of the group (i.e., both in the low and high cognitive load group). Last, based on previous findings that experiencing a traumatic event may prevent the correct identification of safety signals and thus may lead to fear responses, we tested whether there was a positive correlation between the CTQ scores and fear

generalization. However, we did not find any correlation between the degree of fear generalization and the total CTQ scores of the participants.

Even though there were significant differential reactions to CSA4+ compared to CSB4- in the conditioning phase, the fear responses were not retrieved until the generalization phase in either of the groups. One explanation might be that, while there was brief fear conditioning, the use of abstract images (i.e., fractals) made it more difficult for participants to retain these fear responses since they were unrelated to real-life objects or situations. All of the studies with long-lasting fear conditioning effects used real-life objects or geometric shapes as stimuli in the experimental design, which may have made it easier for participants to attach emotional meanings to them (Dunsmoor et al., 2015; Wang et al., 2021; White & Davey, 1989). Another explanation could be that the absence of reinforcement of the previously conditioned stimulus (CSA+) with a shock during the generalization phase led the conditioned response to disappear quickly. It has been demonstrated that when CS was presented without the US, the fear responses faded away (King et al., 2017). Moreover, it was found that the rate of fear extinction was not well predicted by the rate of fear conditioning (King et al., 2017). This finding implies that even when there is high fear conditioning, the fear responses can fade away quickly. Thus, in our case, the lack of fear retention in the generalization phase could be due to the absence of the reinforcement of the conditioned stimulus, i.e., CSA4+.

Moreover, there was no fear generalization to other stimuli on the reinforced sequence (i.e., CSA1, CSA3, and CSA3). The findings from a previous fear generalization study suggested that fear retrieval could be a prerequisite for generalizing fear responses to other stimuli (Roche et al., 2008). Their results showed that fear extinguishes quickly to stimuli that have indirectly acquired the fear responses, i.e., through fear generalization. Hence, it is

possible that the lack of fear generalization in our study was due to the absence of fear retention.

However, another explanation for the lack of fear retrieval and the lack of fear generalization might be that the participants were not explicitly aware of the US-CS contingencies. Accordingly, when asked about shock contingencies, 6 participants said they did not know which CS was followed by a shock. This could have also prevented fear retention and thereby also fear generalization. In line with this hypothesis, previous research found that contingency awareness plays a role in fear retention and generalization (Tabbert et al., 2010). Additionally, researchers found that, compared to participants who were not explicitly aware of the US-CS pairings, those who were aware showed higher fear responses. To exclude this possible effect of shock contingency awareness on fear responses and thus on fear generalization, we calculated a rmANOVA only with participants who correctly identified shock-stimulus pairings. As the results were not statistically significant, we can conclude that explicit knowledge of the shock-stimulus pairings did not affect fear generalization in our study.

In addition, to rule out the possibility that the lack of generalization was due to a failure to explicitly remember the correct order of the stimuli within the sequence, participants took an order memory test. 36 out of 38 participants correctly recalled the order of the images. Subsequently, we ran a rmANOVA for fear generalization with only participants who reported the correct order. The results were not significant; thus, explicit learning of the correct order did not influence fear generalization.

Last but not least, it is probable that we failed to detect a correlation between the CTQ scores and the fear generalization, as there was no fear generalization to begin with. Even if a

correlation had been there, we would have failed to detect it since one of the variables on which the hypothesized correlation was predicated, namely fear generalization, was absent.

Going forward, the experiment can be improved by using real-life objects instead of fractal images to serve as stimuli, considering that it might make it easier for participants to attribute emotional significance to them. Additionally, for future studies, it would be beneficial to first employ a paradigm with no cognitive load and a large enough sample size to capture the effects of inferential fear generalization alone. This would allow them to focus on devising a paradigm that successfully assesses inferential fear generalization. Only then the cognitive load should be incorporated into the paradigm.

There were some limitations to this study. The first limitation of the study was the sample size. Due to time restrictions, collecting 30 participants in each group was not possible as calculated a priori. Instead, data consisted of 39 valid responses to be analyzed. Additionally, several participants were eliminated to assess the influence of forming explicit memory associations, as confirmed by the results of the memory tests. The second limitation was that each experimenter collected an unequal number of participants. As giving compensation for signing up for the study made it easier to recruit participants, one experimenter ended up collecting more participants. Because sample size differences influence the statistical power of ANOVA, it is preferable for sample sizes to be as similar as possible. Thus, it would be ideal to continue data collection until meeting the pre-estimated sample size calculations and considering the unequal data collected by different experimenters and the number of participants to be excluded based on memory test results.

5. Conclusion

Our experiment was the first to apply a sensory pre-conditioning paradigm with sequences to investigate inferential fear generalization. This experimental design allowed us to assess the degree to which fear generalized to stimuli within a sequence. It was also the first study to investigate whether cognitive load influenced this process and whether inferential fear generalization correlated with childhood trauma. Our results showed successful fear conditioning. However, there was no fear retention during the generalization phase, and we did not find any fear generalization. The effects of cognitive load manipulation were likewise insignificant since there was no fear generalization independently of the cognitive load group. Finally, we did not find any correlation between childhood trauma and fear generalization, possibly due to the lack of fear generalization. Nonetheless, the results of this study provide the groundwork for future research into the inferential fear generalization process by improving the experimental design and controlling for potential confounds.

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