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# No influence of threat uncertainty on fear generalization

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## Abstract

Fear overgeneralization and perceived uncertainty about future outcomes have been suggested as risk factors for clinical anxiety. However, little is known regarding how they influence each other. In this study, we investigated whether different levels of threat uncertainty influence fear generalization. Three groups of healthy participants underwent a differential fear conditioning protocol followed by a generalization test. All groups learned to associate one female face (conditioned stimulus, CS+) with a female scream (unconditioned stimulus, US), whereas the other face (CS-) was not associated with the scream. In order to manipulate threat uncertainty, one group (low uncertainty,  $n = 26$ ) received 80%, the second group (moderate uncertainty,  $n = 32$ ) received 60%, and the third group (high uncertainty,  $n = 30$ ) 40% CS-US contingency. In the generalization test, all groups saw CS+ and CS- again along with four morphs resembling the CSs in steps of 20%. Subjective (expectancy, valence, and arousal ratings), psychophysiological (skin conductance response, SCR), and visuocortical (steady-state visual evoked potentials, ssVEPs) indices of fear were registered. Participants expected the US according to their reinforcement schedules and the discriminative responses to CS+/CS- increased with more uncertainty in skin conductance. However, acquisition of conditioned fear was not evident in ssVEPs. During the generalization test, we found no effect of threat uncertainty in any of the measured variables, but the strength of generalization for threat expectancy ratings was positively correlated with dispositional intolerance of uncertainty. This study suggests that mere threat uncertainty does not modulate fear generalization.

## KEYWORDS

fear generalization, intolerance of uncertainty, skin conductance, steady-state visual evoked potentials, threat uncertainty

## 1 | INTRODUCTION

Learned associations between stimuli support the selection of an appropriate and prompt response in the face

of danger and can support successful prediction of future threats. The uncertainty tied to novel situations can be reduced by generalizing information from past experiences to the new one. Fear generalization can help an

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organism to survive potential danger, but overgeneralization can lead to excessive defensive responses and has been implicated in anxiety-related psychopathology (Cha et al., 2014; Greenberg et al., 2013; Lissek et al., 2005, 2008, 2010). Research on threat generalization is often conducted with differential fear conditioning paradigms (Lonsdorf et al., 2017) including two phases: First, participants see two stimuli on the screen and learn to associate one of them (CS+ or threat signal) with an aversive unconditioned stimulus (US), whereas the other stimulus is never associated with the US (CS− or safety signal). After this phase, the CSs along with a set of new generalization stimuli (GS) that lie in a continuum of perceptual resemblance from the CS+ to the CS− are presented. Healthy controls often show a steep generalization gradient, whereas anxious patients often exhibit a wider gradient (Lissek et al., 2014). The latter being coined as an indicator of over-generalization, as innocuous GSs that merely resemble the CS+ still evoke conditioned fear. Responses to the CSs and GSs have been measured using threat ratings (Ahrens et al., 2016; Lemmens et al., 2021; Lissek et al., 2009; Tinoco-González et al., 2015; Wong & Lovibond, 2017), psychophysiological measures such as fear-potentiated startle response (Andreatta et al., 2015; Lissek et al., 2009, 2010), skin conductance response (SCR; Ahrens et al., 2016; Dunsmoor et al., 2017; Herzog et al., 2021; Lemmens et al., 2021; Wong & Lovibond, 2017), steady-state visual evoked potentials (ssVEPs; McTeague et al., 2015; Stegmann et al., 2020), heart rate (Ahrens et al., 2016), as well as brain imaging (Cha et al., 2014; Greenberg et al., 2013). However, it is not yet entirely clear why these differences in generalization responses between patients and healthy individuals exist and why they have been found in some disorders such as panic disorder and post-traumatic stress disorder (Kaczurkin et al., 2017; Lissek et al., 2010, 2014), but evidence remains mixed for others such as generalized anxiety disorder and social anxiety disorder (Ahrens et al., 2016; Lissek et al., 2014; Tinoco-González et al., 2015).

One reason for overgeneralized defensive responses in patients could be poor threat-safety discrimination. Indeed, several recent models highlight the difficulty of patients with pathological anxiety to determine safety (Brosschot et al., 2018; Sangha et al., 2020; Tashjian et al., 2021). Notably, studies reporting group differences in fear generalization often show smaller threat vs. safety discrimination learning in people with clinical anxiety already before the generalization test (Lissek et al., 2010, 2014). This discrimination deficit is also manifested in less discriminate activation in response to threat and safety signals in ventromedial prefrontal cortex (vmPFC), a brain area which is involved in fear inhibition (Cha et al., 2014; Greenberg et al., 2013; Huggins et al., 2021; Milad et al., 2007; Tashjian et al., 2021),

and it could, therefore, reflect an overall difficulty in evaluating how safe a stimulus is.

According to Tashjian et al. (2021), perceived safety is not simply the exact opposite of threat perception, but instead includes distinct computations by incorporating both threat- and self-related evaluations. This model suggests that one of the determinants of safety perception is uncertainty about the predictability of threat. Although unpredictability and uncertainty share many characteristics, there is an important distinction. Threat unpredictability is often referred to as the objective probability of an aversive event to occur and it has been considered central in inducing anxiety (Grupe & Nitschke, 2013). Unpredictable threat has been shown to increase vigilance (Kastner-Dorn et al., 2018; Wieser, Reicherts, et al., 2016) and startle sensitivity (Grillon et al., 2008) both in clinical and healthy samples and is associated with biased expectations of threat (Grupe & Nitschke, 2011; Sarinopoulos et al., 2010). On the other hand, uncertainty refers to the subjective difficulty to predict a future outcome (Grupe & Nitschke, 2013). Several recent models consider uncertainty central in anxiety psychopathology (Brosschot et al., 2016; Carleton, 2016; Carleton et al., 2012; Grupe & Nitschke, 2013).

One of these models concerns intolerance of uncertainty, the dispositional tendency to find ambiguous or uncertain events aversive (Carleton, 2016; Carleton et al., 2012). Several studies have examined the relationship between trait intolerance of uncertainty and fear generalization with inconsistent outcomes: Morriss et al., (2016) used a fear conditioning paradigm, which included the GS already in the acquisition while the CS+ was reinforced 50% of the time. They found more generalization of skin conductance responses to the test stimuli in acquisition for participants scoring high in intolerance of uncertainty and delayed extinction of uneasiness ratings, but these results have not been consistently replicated (Bauer et al., 2020). Regardless of the inconsistent findings, fear conditioning paradigms that present the GS already in acquisition make it difficult to differentiate between generalization of a response associated with threat to a novel stimulus and impaired fear learning. Another study examined whether intolerance of uncertainty, along with other anxious traits, correlates with conceptual fear generalization gradients but found no correlation with intolerance of uncertainty (Mertens et al., 2021). Therefore, it seems that trait intolerance of uncertainty affects to some extent stimulus discrimination. However, evidence is mixed and no other studies, to our knowledge, have examined the role it plays in differential fear conditioning with a generalization test.

Despite the dispositional intolerance toward uncertain events, external factors can also influence how accurately someone can predict a threat and how well they can discriminate between threat and safety.

State uncertainty regarding threat can be manipulated through learning about threat contingencies (Tashjian et al., 2021). Protocols with higher reinforcement rates (i.e., 100%) give participants more chances to learn the conditions in which the threat occurs and thus have the potential to make the occurrence of threat more predictable than with partial reinforcement (i.e., 50%). Partial reinforcement schedules have been documented to lead to impaired extinction learning (Dunsmoor et al., 2017; Grady et al., 2016; Grant & Schipper, 1952; Jenkins & Rigby, 1950; Nevin, 1988; Pittenger & Pavlik, 1988), but also partial reinforcement schedules such as 50% have been shown to potentiate startle conditioned responses the higher the trait intolerance of uncertainty is but not during a 75% reinforcement schedule (Chin et al., 2016). In fact, partial reinforcement is found to involve distinct patterns of brain activity compared with continuous reinforcement schedules, which are hypothesized to reflect the uncertainty induced by partial reinforcement (Dunsmoor et al., 2007).

Despite the inherent uncertainty associated with partial reinforcement rates, studies on fear generalization use various reinforcement schedules during acquisition, ranging from 33% (Morey et al., 2015) to 75% or even 100% (Lemmens et al., 2021; Lissek et al., 2010) making it difficult to compare. To our knowledge, the only study that directly investigated the effect of partial and continuous reinforcement schedules on fear generalization is the one by Zhao et al. (2022). The authors compared three groups with reinforcement schedules of 50%, 75%, and 100% in acquisition and found overall increased generalization magnitudes for threat expectancy ratings for the groups with partial (50% and 75%) reinforcement, whereas the continuous reinforcement group showed a less steep generalization gradient. Surprisingly, no effect of the reinforcement rate was evident in SCR during acquisition and generalization despite some evidence that SCR is modulated by US prediction (de Berker et al., 2016; Ojala & Bach, 2020). However, Zhao et al. (2022) used a 50% reinforcement schedule for all groups in generalization, which matched the reinforcement schedule of one group (i.e., the 50% group) during acquisition. This means that each group experienced a different reduction (whereas none for the 50%) of CS-US contingency, making the generalization test difficult to compare across groups. Therefore, state uncertainty from partial reinforcement schedules seems to increase the expectancy of threat in generalization, but these results are hindered by a different reduction from acquisition to generalization phase for the three groups.

There is a variety of factors that interact in fear generalization: from threat detection and threat versus safety discrimination to the selection of the correct behavioral response. This multifaceted nature of fear generalization

is reflected in the dissociations found between different measures such as visuocortical (McTeague et al., 2015) and fear-potentiated startle (Lissek et al., 2008), heart rate and SCR (Ahrens et al., 2016), US expectancy and SCR (Lemmens et al., 2021). Notably, although most measures used to study fear generalization show either a quadratic or linear gradient with stronger responses to CS+ and decreasing responses along the stimulus dimension as the stimuli resemble more the CS-, the visual cortex shows a different function. McTeague et al. (2015) used ssVEPs to investigate the involvement of the visual cortex in early bias formation and fear generalization. SsVEPs is an oscillatory response to luminance modulated stimuli (i.e., flickered) in which the electrocortical response recorded from the scalp resonates at the same frequency as the driving stimulus (Norcia et al., 2015; Regan, 1966). Enhanced attention to the driving stimulus is associated with increased ssVEP amplitude (Vialatte et al., 2010; Wieser, Miskovic, et al., 2016), and it has been reported for visual stimuli associated with threat in fear conditioning studies (Miskovic & Keil, 2013; Moratti & Keil, 2005). Using a fear generalization paradigm, McTeague et al. (2015) found a response that resembled a pattern of lateral inhibition with the lowest response to the stimulus closest in resemblance to the CS+. The authors suggested that this pattern might exhibit visual cortex's action to discriminate between a stimulus that signals threat (CS+) and another similarly looking but new stimulus (GS1). The same pattern has been observed in other neuroimaging and electrophysiology studies (Friedl & Keil, 2021; McTeague et al., 2015; Onat & Büchel, 2015; Stegmann et al., 2020). The different generalization gradients found in the different systems involved in fear generalization might reflect that each system has a distinct function. This multifaceted nature of fear generalization in combination with inconsistent findings regarding its role in anxiety disorders makes evident the need for further investigation in the manifestations of fear generalization and the factors that modulate it.

To this end, in the present study, we examined whether state threat uncertainty defined as the frequency in which the CS+ predicts US onset would lead to overgeneralization of conditioned responses. Threat uncertainty was manipulated by creating different CS+/US contingencies in three different groups. More specifically, the group with low uncertainty (LU) received 80%, the one with moderate uncertainty (MU) received 60%, and the one with high uncertainty (HU) only 40% reinforcement rate. Since fear generalization is a multifaceted response, we included four different response measures to see how it is manifested in different psychophysiological, affective, and cognitive measures. To this end, we recorded ssVEPs, SCR and ratings of valence, arousal, and US expectancy. Although only one study to date has investigated the influence of

threat uncertainty on generalization (Zhao et al., 2022), based on the aforementioned literature, we expected that increasing uncertainty will lead to less steep (i.e., more linear) generalization gradients. Furthermore, since fear generalization is manifested differently in different measures, we had separate expectations. For the ssVEPs, we predicted that in the LU group, the lateral inhibition model will be evident, which will be less prominent the more uncertainty increases (in the MU and HU groups). We further predicted that the SCR and the ratings will be modulated by threat uncertainty such that in the HU group, participants will transfer their responses from CS+ to a wider range of GSs than in the MU and LU groups and that participants in the MU group will transfer their responses to a wider range of GSs than in the LU group.

## 2 | METHOD

### 2.1 | Participants

A total of 95 undergraduate students at the Erasmus university Rotterdam were recruited in exchange for course credit. Based on previous literature (Kastner et al., 2015; Stegmann et al., 2020; Talmi et al., 2019), the smallest effect size we expected was for ssVEPs for the comparison between CS+ and CS-. We used G\*power 3.1.9.7 to calculate the interaction between the two CSs and the three groups, with a small to moderate effect size  $f = .20$ , alpha set at .05 and power at .85. The power analysis indicated a total sample of 72 participants would be sufficient; however, we collected 95 to account for exclusions due to technical problems. Seven participants were excluded (two from the high-uncertainty group and five from the low-uncertainty group) due to technical problems, resulting in

a final sample of 88 (17 male, 1 non-binary) with a mean age of 20.54 years ( $SD = 3.45$ ). All participants had normal or corrected-to-normal vision and no family history of photic epilepsy. Participants were randomly assigned to one of the three groups. The groups did not differ in age, sex ratio, depression, state anxiety, or intolerance of uncertainty levels (see Table 1). However, the LU group had higher levels of trait anxiety and social anxiety than the MU group. More information regarding the ethnicity of the participants included in the study can be found in Table S1 of the supplementary materials. The experimental procedure of the current study was approved by the Ethical Committee of the Erasmus University Rotterdam in accordance with the declaration of Helsinki. The study has been registered in Open Science Framework ([https://osf.io/tdqj3/?view\\_only=fb8ca7beabb444d38aadd8a999249a44](https://osf.io/tdqj3/?view_only=fb8ca7beabb444d38aadd8a999249a44)).

### 2.2 | Materials

#### 2.2.1 | Stimuli

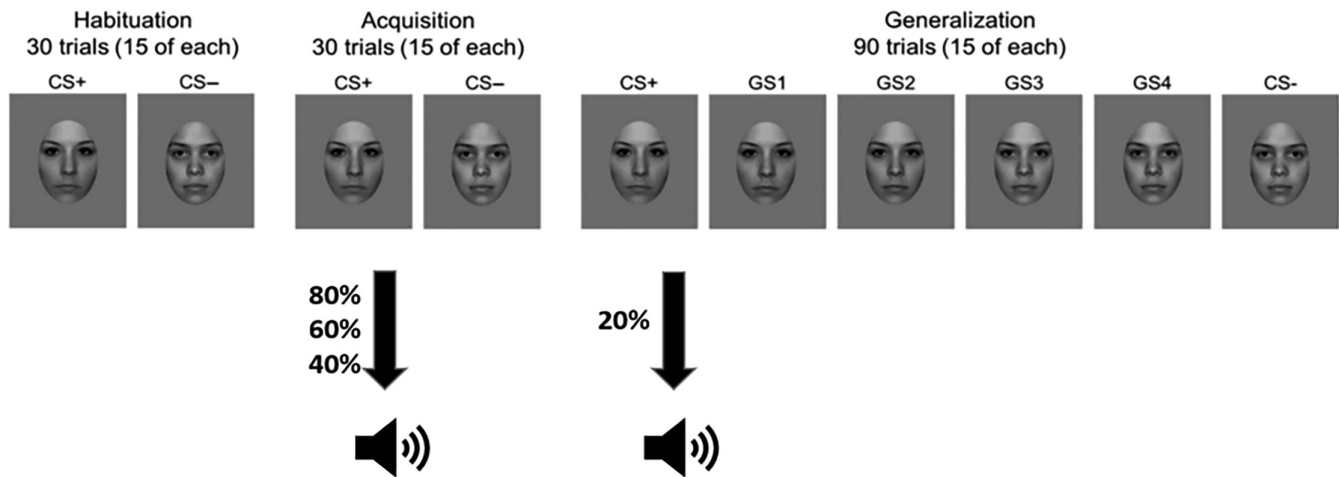
The CS were two pictures of female faces with a neutral facial expression from the NimStim Set of Facial Expressions (03F\_NE\_C, 10F\_NE\_C; Tottenham et al., 2009). The images were converted to gray scale, and luminance and brightness were kept constant. Four GSs were morphed from the CS pictures in steps of 20% with a face-morphing software shown in Figure 1 (Squirrelz Morph; Xiberpix, Solihull, UK). Each picture was presented on a gray background (R: 133, G: 133, B:133) flickering at 15 Hz. The US was a female scream combined with a white noise of 90 dB that lasted 1 s and was presented through four field speakers.

**TABLE 1** Descriptive statistics for the different groups.

Variable	HU	MU	LU	$F/\chi^2$	p-value	Possible range
<i>N</i>	30	32	26			
Sex	F:26 M:4	F:22 M:10	F:22 M:3	6.86 (4)	.144	
	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>			
Age	20.67 (4.72)	20.47 (2.07)	20.23 (2.97)	0.11 (2, 85)	.894	
STAI-S	43.27 (5.09)	44.88 (6.05)	44.85 (5.19)	0.83 (2, 85)	.438	20–80
STAI-T	47.63 (4.40)	46.09 (3.73)*	49.15 (4.62)*	3.74 (2, 85)	.028*	20–80
IUS	60.83 (19.48)	58.72 (16.87)	62.85 (21.21)	0.34 (2, 85)	.716	27–135
LSAS	38.77 (19.44)	33.84 (19.97)*	48.65 (25.28)*	3.46 (2, 85)	.036*	0–144
BDI-II	8.47 (6.80)	9.31 (5.98)	11.50 (10.58)	1.09 (2, 85)	.341	0–63

Abbreviations: BDI-II, Beck's Depression Inventory-II; IUS, Intolerance of Uncertainty Scale; LSAS, Liebowitz Social Anxiety Scale; STAI-S, State Trait Anxiety Inventory-State; STAI-T, State Trait Anxiety Inventory-Trait.

\* $p < .05$ .



**FIGURE 1** Experimental procedure and conditioning protocol. In the habituation phase, no US was presented. In acquisition, the reinforcement rate for the CS+ US coupling was different for each group (40%, 60%, and 80%). In Generalization, the CS+ was reinforced at a 20% rate in all groups.

### 2.2.2 | Questionnaires

Four questionnaires were used to measure participants' underlying psychopathological traits. Depression levels were assessed with the second edition of Beck's Depression Inventory (BDI-II; Beck et al., 1961; Dutch translation: Does, 2002). BDI-II contains 21 items, each corresponds to a symptom of depression and measured in a four-point-scale (0–3). Anxiety levels were measured with State–Trait Anxiety Inventory (STAI; Spielberger, 1970; Dutch translation: Ploeg, 2000), which contains 20 items measuring state anxiety (STAI-S) and 20 items measuring trait anxiety (STAI-T). The items are measured in a four-point scale (1 = *Not at all/Almost never*, 4 = *Very much so/Almost always*). Intolerance of uncertainty was measured with the Intolerance of Uncertainty Scale (IUS; (Buhr & Dugas, 2002; Dutch translation: de Bruin et al., 2006), which is a 27-item scale that measures the dispositional tendency to find uncertain situations aversive and anxiety provoking (Morris et al., 2016). IUS' items are measured in a 5-point scale ranging from 1 = *not at all characteristic of me* – 5 = *entirely characteristic of me*. Social anxiety levels were measured with Liebowitz Social Anxiety Scale (LSAS; Liebowitz, 1987) that includes 24 items, each assessing both fear or anxiety and avoidance behaviors. The items are measured in a 4-point scale ranging from 0 = *non/never* to 4 = *severe/usually*.

### 2.2.3 | Ratings

During the experiment, we obtained participants' valence and arousal ratings of the stimuli, using the Self-Assessment Manikin scale (SAM; Bradley & Lang, 1994).

Both valence and arousal scales range from 1 “very pleasant” (valence) or “very calm” (arousal) to 9 “very unpleasant” (valence) or “very arousing” (arousal). Furthermore, we measured US expectancy, and after the generalization test, we employed a visual discrimination task of the stimuli. Participants used the arrow keys in the keyboard to indicate their affective responses and then pressed “enter.” US expectancy was measured with the question “How likely is this face to be followed by a scream?” presented along with each face. Participants could give an answer ranging from 0 to 100 by dragging a red bar to the most appropriate point in the scale with their cursor. Finally, we measured participants' ability to discriminate the different stimuli to further explore whether the responses in the generalization phase were due to inability to perceptually differentiate between the different stimuli (Resnik et al., 2011; Struyf et al., 2017). Discrimination was assessed in five trials, by comparing each face with the CS+. In each trial, two stimuli (CS+ and one of the other pictures) were presented one by one (no interstimulus interval) for 1 s each in a random order. After each presentation, participants answered whether the two pictures showed the same face by pressing either “y” for yes or “n” for no using the keyboard. All ratings' questions were presented for an indefinite amount of time until a response was given.

### 2.3 | Study design and procedure

After signing the informed consent and completing the questionnaires, participants were seated in a reclining chair in a separate soundproof EEG room, where the EEG and SCR electrodes were applied. The seat was positioned

1.5 meters away from a 22-inch iiyama HM204DT-A computer screen with 120 Hz refresh rate. Participants were instructed to remain as motionless as possible during the experiment and were informed that they will see two faces, but only one will sometimes be followed by a loud scream. The experiment included three phases: Habituation, Acquisition, and Generalization. In the habituation phase, participants saw the CS+ and CS− for 10 times each, without the US (see [Figure 1](#)). In the Acquisition phase, each CS was presented 15 times and only the CS+ was followed by the US at the offset of the CS+. The reinforcement rate differed between groups (LU: 80%, MU: 60%, HU: 40%). The following Generalization phase was identical for all the groups, in which all six faces were presented (15 times each). That means the four GSs and the CSs were presented 15 times each. The CS+ was reinforced 20% of the time to minimize extinction (Lissek et al., 2008). All faces flickered at 15 Hz for 5 s, to evoke ssVEPs. All stimuli were presented in a pseudorandomized order so that the same faces were not presented more than twice in a row and that the Acquisition phase always started with a CS+ presentation for all groups. Each stimulus was presented for 5 s and the US appeared at the CS+ offset. The duration between one stimulus offset and the next stimulus onset termed Inter-Trial Interval (ITI) ranged from 9 to 10 s.

Valence and arousal ratings were measured at the end of each phase, and US expectancy was measured at three time points: half-way through Acquisition, at the end of Acquisition, and at the end of Generalization. Discrimination of stimuli was tested only at the end of the Generalization phase to avoid priming the participants about the number of different faces presented. The whole task lasted approximately 45 min.

## 2.4 | Psychophysiological recording and analysis

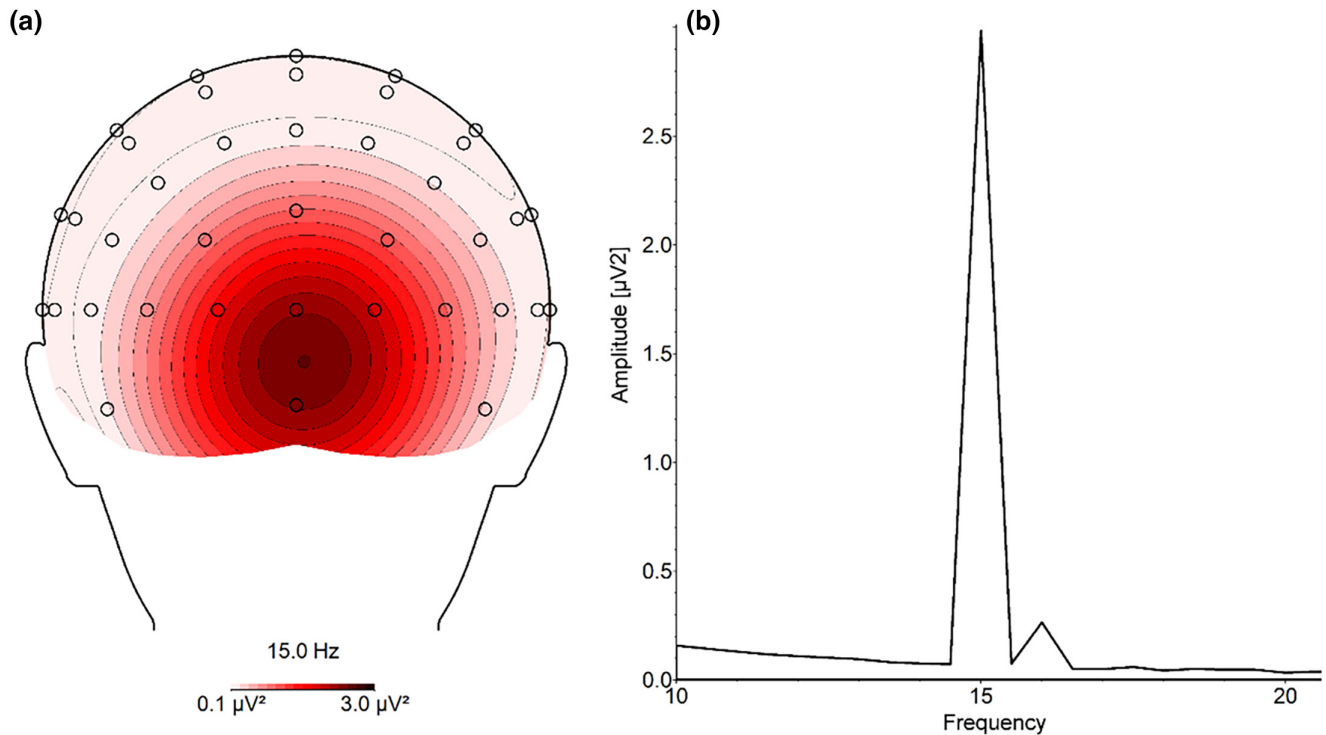
Brain activity was recorded with electroencephalogram (EEG) using the Biosemi Active-Two amplifier system (Biosemi, Amsterdam, the Netherlands) from 64 Ag/AgCl active electrodes attached to an elastic cap according to the 10/20 system. The Biosemi Active-Two system includes two extra electrodes instead of a single ground electrode, namely Common Mode Sense (CMS) and Driven Right Leg (DRL), which act as online reference and ground. In addition, the electro-ocular activity (EOG) was recorded with two flat type active electrodes that were placed on the two outer canthi of both eyes to record horizontal eye activity and two more were placed on the infraorbital and a supraorbital region of the right eye to register eye vertical movements. The signal was digitized at a 512 Hz sampling

rate, and 24-bit analog-to-digital conversion and threshold of impedance was kept below 30 k $\Omega$ .

SCR was recorded using the same Biosemi Active-Two amplifier system. Two 8-mm Ag/AgCl electrodes that contained 0.05M NaCl electrolyte medium were attached to the second phalanx of the middle and ring finger of the non-dominant hand after being lightly cleaned with water.

Both EEG and SCR were analyzed offline with the software BrainVision Analyzer 2.0 (BrainProducts Inc., Gilching, Germany). For the continuous EEG recordings, data were filtered offline with 0.1 Hz low cut-off, 40 Hz high cut-off, and 50 Hz notch filters. Ocular artifacts were detected and corrected with Gratton-Cole artifact correction procedure (Gratton et al., 1983). Thereafter, data were re-referenced to the average of all electrodes and segmented into time windows of 500 ms pre-stimulus until 5500 ms after stimulus onset. Artifacts were rejected according to the following criteria: (1) maximal amplitude allowed was 200  $\mu$ V, and (2) lowest activity allowed in intervals was 0.5  $\mu$ V (100 ms interval length), and subsequently the remaining trials were averaged according to the experimental conditions. The signal was then manually inspected for artifacts, but no irregularities were observed. Afterwards, the signal was transformed into the frequency domain using a fast Fourier transformation for the last 2000 ms of stimulus presentation in order to eliminate initial non-stationary ssVEP components and to highlight ssVEP power, which has shown to be more sensitive to conditioning effects in the second half of CS presentation (Miskovic & Keil, 2013; Moratti et al., 2006; Moratti & Keil, 2005). Based on previous studies (Stegmann et al., 2020; Wieser et al., 2014) and the topography (see [Figure 2](#)), the ssVEP signal was averaged across electrodes Iz, Oz, O1, and O2, and mean activity from these electrodes was used in the statistical analyses. Both reinforced and unreinforced trials were included in the analysis.

SCR was filtered offline with a high cut-off of 1 Hz and the signal was segmented in epochs of −1000 to 8000 ms after stimulus onset. The signal was quantified using a manual Foot-to-Peak method with a latency time-window of 900–4000 ms after stimulus onset (Boucsein, 2012). We considered the first response peak following the foot located at 500 ms after the foot point until the end of the time window. Responses below 0.02  $\mu$ S were scored as zero. Finally, SCRs were log-transformed ( $\log [1 + \text{SCR}]$ ) to normalize the distribution and mean responses were calculated per condition and phase. We decided not to exclude non-learners based on SCR for two reasons: First, SCR is not a direct measure of learning and excluding participants solely on this can lead to sample bias (Lonsdorf et al., 2019). Second, since our experimental manipulation



**FIGURE 2** Topography of mean 15 Hz ssVEP activity averaged across stimuli in Habituation and Acquisition (a) and the corresponding FFT power at electrode Oz (b). The signal shows the involvement of the visual cortical areas at the driving frequency of 15 Hz.

included different reinforcement schedules, smaller SCR amplitude could be due to the experimental manipulation.

## 2.5 | Statistical analyses

All statistical analyses were carried out in RStudio (Version 4.1.2, RStudio Team, 2021). In contrast to the pre-registration of this study, we analyzed the data with linear mixed models instead of rm ANOVA, which is often used in generalization research. This decision was based on several limitations of rm ANOVA including the assumption of sphericity, which is often unmet for generalization data (Vanbrabant et al., 2015). Instead, linear mixed models have fewer assumptions and offer a more reliable statistical inference (Vanbrabant et al., 2015). Furthermore, there is a rise of linear mixed models use in generalization research the last years (Ginat-Frolich et al., 2019; Struyf et al., 2018).

Linear mixed models were conducted separately for each experimental phase with SCR, ssVEPs, valence, arousal, and US expectancy as separate dependent variables. These models were fitted using the packages lme4 and lmerTest (Bates et al., 2015; Kuznetsova et al., 2017), and significance is reported with the Kenward-Rogers approximation for the degrees of freedom (Kenward & Roger, 1997). All analyses included the intercept of the Participants as a random effect. For habituation, Stimulus (CS+ and CS-) and Group

(LU, MU, and HU) were fixed factors. In acquisition, the ratings of valence and arousal were analyzed in the same manner as in habituation. For ssVEPs, SCR, and US expectancy, Stimulus, Time (Acq1 for the first half of Acquisition, Acq2 for the second half of Acquisition), and Group were fixed factors. Significant interactions were followed up with planned contrasts on the development of the differential stimulus responding from Acquisition 1 to Acquisition 2 for all group comparisons (LU-HU, LU-MU, and HU-MU). For the factors Time and Stimulus, Acquisition 1 and CS- were the reference levels, respectively. In generalization, Stimulus and Group were entered as fixed factors, but this time Stimulus had six levels (CS+, GS1, GS2, GS3, GS4, and CS-). Significant main effects for Stimulus were followed up with simple contrasts models with CS- as reference point (Lissek et al., 2008). In case of significant interactions with the factor Group, we further described the shape of the gradients with trend analyses. Specifically, we assessed whether the gradients differed in terms of linearity or curvature across groups. To this end, two orthogonal polynomial trend repeated measures contrasts across all test stimuli served as fixed factors to examine the shape of generalization gradient. Specifically, linear trend repeated measures contrast assessed a monotonic gradient across all test stimuli, whereas the quadratic trend assessed curvature gradients. In order to control for time effects during the generalization phase, we exploratively tested the effects of uncertainty with regard to the first and second half of the



generalization phase for the SCR and ssVEPs. This analysis did not yield any significant interaction effects with Time (all  $p$  values  $>.399$ ) so we did not include it in the results section.

We followed the frequentist analysis for the generalization phase with Bayesian analysis to evaluate the evidence for the null hypothesis. We calculated Bayes factors ( $BF_{10}$ ) using the package BayesFactor (version 0.9.12-4.4) with default JZS priors (Jarosz & Wiley, 2014; Rouder et al., 2012). Additionally, in order to isolate the evidence for the effect of the experimental manipulation on stimulus generalization (Stimulus  $\times$  Group interaction), we used inclusion Bayes factors ( $BF_{inclusion}$ ; Clyde et al., 2011) with the package bayestestR (version 0.13.1). Here, inclusion Bayes factors quantify the evidence for the desired effect, the Stimulus  $\times$  Group interaction, by averaging the evidence for all models including the effect versus the average of all models excluding the effect. Both the  $BF_{10}$  (null: random intercepts for the participants) and the  $BF_{inclusion}$  for all main effects and interactions are reported. According to the criteria for interpreting Bayes factors by Jeffreys (1961) and Lee and Wagenmakers (2013), we consider Bayes factors  $>10$  to indicate strong evidence for the alternative hypothesis/for inclusion and Bayes factors  $<0.10$  to indicate strong evidence for the null hypothesis/against inclusion.

Furthermore, we quantified the strength of the generalization with a linear deviation score ( $([GS1, GS2, GS3, GS4] / 4) - ([CS+, CS-] / 2)$ ; LDS). The LDS is a single number representing the steepness and strength of the generalization gradient. Positive values correspond to shallow and stronger generalization gradients, whereas negative values correspond to steeper and weaker generalization gradients (Berg et al., 2021; Kaczurkin et al., 2017). LDS scores of each group for each measure were compared with one-way ANOVAs with LDS as dependent variable and Group as between-subjects factor. Finally, as an exploratory analysis, we correlated the sum of scores of the IUS with the LDS in order to explore if dispositional intolerance of uncertainty played a role in participants' generalized responses. Additionally, we investigated whether participants in the three groups differed in how well they discriminated between CS+ and the other test stimuli. Participant's responses were transformed to 1 (accurate response) and 0 (inaccurate response). We then calculated the average discrimination response per participant for the five comparisons and calculated between-groups differences for the three groups with one-way ANOVAs. For the analysis of the discrimination task, eight participants were further excluded due to equipment failure, resulting in 80 participants included in this analysis. For all statistical analyses, alpha level was set at .05 and Bonferroni correction was used to adjust the alpha level for multiple comparisons.

## 3 | RESULTS

### 3.1 | Habituation

#### 3.1.1 | Psychophysiology

There were no significant main or interaction effects in habituation for SCR (all  $p$  values  $>.246$ ) and ssVEPs (all  $p$  values  $>.222$ ), indicating that there was no preferential processing of either of the CSs in any of the groups.

#### 3.1.2 | Ratings

Similar to the psychophysiological responses, the linear mixed models for valence (all  $p$  values  $>.335$ ) and arousal ratings (all  $p$  values  $>.391$ ) returned no significant effects. Means and standard deviations for all variables in Habituation can be found in Table 2.

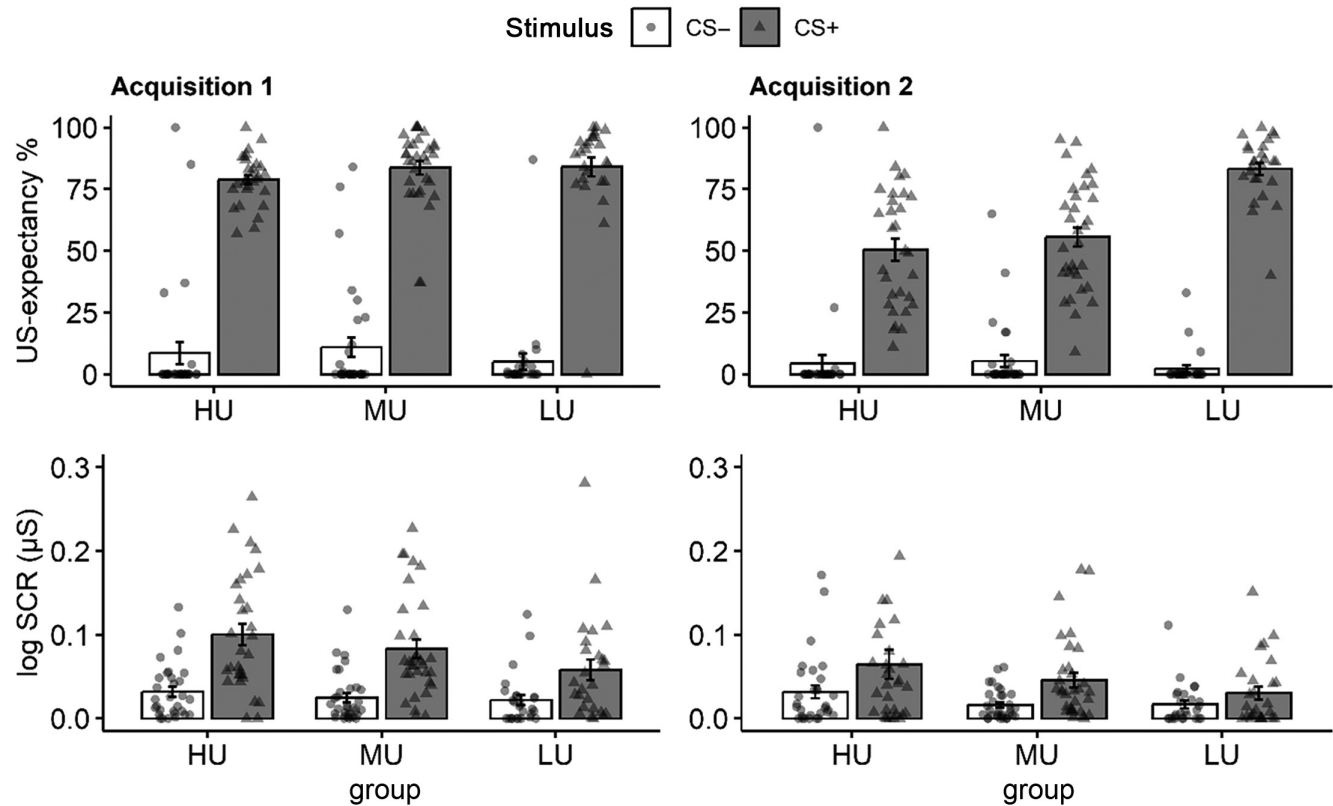
### 3.2 | Acquisition

#### 3.2.1 | Psychophysiology

During acquisition, the SCR showed a significant Stimulus  $\times$  Time interaction,  $F(1,255)=13.99$ ,  $p<.001$ ,  $R^2=.114$ , and a significant Stimulus  $\times$  Group interaction,  $F(2,255)=3.71$ ,  $p=.026$ ,  $R^2=.024$ , but the Stimulus  $\times$  Group  $\times$  Time interaction was not significant,  $F(2,255)=0.23$ ,  $p=.792$ ,  $R^2=.002$ . Follow-up analyses showed that the difference between CS+ and CS- was larger in group HU compared with LU,  $b_{Group(HU-LU)*Stimulus}=-0.02$ ,  $SE=0.01$ ,  $t(261.00)=2.48$ ,  $p=.014$ , but there was no difference between groups HU-MU,  $b_{Group(HU-MU)*Stimulus}=-0.007$ ,  $SE=0.01$ ,  $t(261.00)=0.67$ ,  $p=.501$  or LU-MU,  $b_{Group(LU-MU)*Stimulus}=0.02$ ,  $SE=0.01$ ,  $t(261.00)=1.87$ ,  $p=.062$  (Bonferroni correction  $\alpha<.017$ ). Furthermore, as shown in Figure 3, averaged across groups, participants showed increased differential SCR responding in Acq1 compared with Acq2,  $b_{Time*Stimulus}=-0.03$ ,  $SE=0.01$ ,  $t(261.00)=3.77$ ,  $p<.001$ .

TABLE 2 Means and standards deviations of the measures during habituation.

Variable	CS+	CS-
	$M$ ( $SD$ )	$M$ ( $SD$ )
Valence (1-9)	5.01 (1.23)	4.97 (1.54)
Arousal (1-9)	4.46 (1.84)	4.56 (1.81)
SCR (microS)	0.02 (0.02)	0.02 (0.02)
ssVEPs (Power FFT)	1.72 (2.21)	1.55 (1.65)



**FIGURE 3** Means and standard errors of the mean for CS+ and CS- in groups high uncertainty (HU), moderate uncertainty (MU), and low uncertainty (LU) depicted for Acquisition 1 (left panel) and Acquisition 2 (right panel) for US expectancy and SCR.

**TABLE 3** Means and standard deviations of the measures during acquisition.

Variable	CS+	CS-
	<i>M</i> ( <i>SD</i> )	<i>M</i> ( <i>SD</i> )
SCR (microS)	0.07 (0.07)***	0.02 (0.03)
ssVEPs (Power FFT)	1.72 (2.24)	1.68 (2.06)
Valence (1-9)	7.42 (1.50)***	4.46 (1.92)
Arousal (1-9)	7.32 (1.82)***	4.20 (2.01)
US-exp (0-100)	72.08 (22.90)***	6.27 (18.40)

\*\*\* $p < .001$ .

For ssVEPs in acquisition, there was no significant main effect of Stimulus or Group (all  $p$  values  $>.331$ ), indicating that participants' visuo-cortical engagement was similar for the two stimuli across the three groups, see Table 3 for means and standard deviations per test stimulus. There was a significant main effect for Time,  $F(1,255) = 8.30$ ,  $p = .004$ ,  $R^2 = .033$  with overall stronger responses in Acq1 ( $M = 1.19$ ,  $SD = 1.40$ ) than Acq2 ( $M = 1.03$ ,  $SD = 1.24$ ). No interaction effects were found (all  $p$  values  $>.393$ ). Consequently, there was no difference in visuo-cortical engagement for either of the two faces and no group differences (HU: CS+:  $M = 1.29$ ,  $SD = 1.34$ , CS-:  $M = 1.19$ ,  $SD = 1.06$ , MU: CS+:  $M = 0.79$ ,

$SD = 0.94$ , CS-:  $M = 0.84$ ,  $SD = 1.00$ , LU: CS+:  $M = 1.30$ ,  $SD = 1.76$ , CS-:  $M = 1.20$ ,  $SD = 1.65$ ), but there was more visuo-cortical engagement to both faces during Acq1 than Acq2.

### 3.2.2 | Ratings

The CS+ was rated more unpleasant and more arousing than CS- (see Table 3 for means and standard deviations),  $F(1,85) = 144.47$ ,  $p < .001$ ,  $R^2 = .630$  and  $F(1,85) = 120.40$ ,  $p < .001$ ,  $R^2 = .586$ , respectively. However, no modulation by threat uncertainty was found for the affective ratings as the main effect of Group and Stimulus  $\times$  Group interaction was not significant (all  $p$  values  $>.219$ ), HU: CS+:  $M = 7.90$ ,  $SD = 1.03$ , CS-:  $M = 4.37$ ,  $SD = 2.12$ , MU: CS+:  $M = 7.19$ ,  $SD = 1.51$ , CS-:  $M = 4.66$ ,  $SD = 1.99$ , LU: CS+:  $M = 7.15$ ,  $SD = 1.85$ , CS-:  $M = 4.35$ ,  $SD = 1.62$ , for valence and HU: CS+:  $M = 7.80$ ,  $SD = 1.83$ , CS-:  $M = 4.43$ ,  $SD = 2.13$ , MU: CS+:  $M = 6.81$ ,  $SD = 1.91$ , CS-:  $M = 4.22$ ,  $SD = 2.18$ , LU: CS+:  $M = 7.42$ ,  $SD = 1.60$ , CS-:  $M = 3.92$ ,  $SD = 1.67$ , for arousal.

The linear mixed models for US expectancy returned a significant Stimulus  $\times$  Group  $\times$  Time interaction,  $F(2,255) = 4.96$ ,  $p < .008$ ,  $R^2 = .037$ . As shown in Figure 3, when compared with LU, the differential

stimulus response in US expectancy decreased from Acquisition 1 to Acquisition 2 for HU,  $b_{\text{Time*Stimulus*Group(LU-HU)}} = -26.06$ ,  $SE = 9.19$ ,  $t(255.00) = 2.83$ ,  $p = .005$  and for MU,  $b_{\text{Time*Stimulus*Group(LU-MU)}} = -24.39$ ,  $SE = 9.06$ ,  $t(255.00) = 2.69$ ,  $p = .008$ . However, this decrease in differential stimulus responding from Acq1 to Acq2 was not significant for the comparison between MU-HU,  $b_{\text{Time*Stimulus*Group(HU-MU)}} = 1.66$ ,  $SE = 8.72$ ,  $t(255.00) = 0.19$ ,  $p = .849$  (Bonferroni correction  $\alpha < .017$ ).

### 3.3 | Generalization

#### 3.3.1 | Psychophysiology

In the generalization phase, the analysis for SCR returned a significant difference between the stimuli,  $F(5, 424.03) = 22.93$ ,  $p < .001$ ,  $R^2 = .213$ ,  $BF_{10} = 2.81e+16$ ,  $BF_{\text{inclusion}} = 1.87e+16$ , but neither a difference between the groups,  $F(2, 85.00) = 1.20$ ,  $p = .307$ ,  $R^2 = .027$ ,  $BF_{10} = 0.006$ ,  $BF_{\text{inclusion}} = 0.004$ , nor a significant Stimulus  $\times$  Group interaction,  $F(10, 424.03) = 0.91$ ,  $p = .528$ ,  $R^2 = .021$ ,  $BF_{10} = 458.16$ ,  $BF_{\text{inclusion}} = 6.49e-14$ . Simple contrast models demonstrated that participants' SCR response to CS- differed significantly as compared to CS+,  $b_{(CS-, CS+)} = 0.03$ ,  $SE = 0.004$ ,  $t(434.00) = 8.24$ ,  $p < .001$ , GS1,  $b_{(CS-, GS1)} = 0.02$ ,  $SE = 0.004$ ,  $t(434.00) = 6.60$ ,  $p < .001$ , GS2,  $b_{(CS-, GS2)} = 0.01$ ,  $SE = 0.004$ ,  $t(434.00) = 2.89$ ,  $p = .004$ , but did not differ to GS3,  $b_{(CS-, GS3)} = 0.00$ ,  $SE = 0.004$ ,  $t(434.00) = 1.07$ ,  $p = .287$ , and GS4,  $b_{(CS-, GS4)} = 0.00$ ,  $SE = 0.004$ ,  $t(434.00) = 0.38$ ,  $p = .706$  (Bonferroni correction  $\alpha < .010$ ). Trend analysis revealed both a significant Linear,  $F(1, 437.00) = 108.72$ ,  $p < .001$  and a Quadratic trend across stimuli and groups,  $F(1, 433.04) = 7.98$ ,  $p = .005$ . The linearity of the overall SCR generalization gradient was characterized by a monotonic decrease from CS+ to CS-, whereas the curvature of the gradient was characterized by a strong generalized responding from CS+ to GS1 and GS2.

For ssVEPs, the main effect of Stimulus just failed to reach the significance level,  $F(5, 425) = 2.15$ ,  $p = .059$ ,

$R^2 = .025$ ,  $BF_{10} = 2.63e-05$ ,  $BF_{\text{inclusion}} = 1.75e-05$ , and there was no main effect of Group,  $F(2, 85) = 1.43$ ,  $p = .244$ ,  $R^2 = .033$ ,  $BF_{10} = 0.011$ ,  $BF_{\text{inclusion}} = 0.007$ , or Stimulus  $\times$  Group interaction,  $F(10, 425) = 1.12$ ,  $p = .341$ ,  $R^2 = .026$ ,  $BF_{10} = 4.05e-19$ ,  $BF_{\text{inclusion}} = 1.60e-18$ , indicating that participants' visuocortical engagement was almost similar for the six stimuli in the three groups. We exploratorily followed the nearly significant main effect of Stimulus since it was one of our main hypotheses. Simple contrast models indicated that only GS2 differed significantly from CS-,  $b_{(CS-, GS2)} = 0.10$ ,  $SE = 0.04$ ,  $t(435.00) = 2.34$ ,  $p = .019$ , but it did not survive Bonferroni correction ( $\alpha < .010$ ). All other stimuli showed no significant differences from CS- (CS+:  $b_{(CS-, CS+)} = 0.03$ ,  $SE = 0.04$ ,  $t(435.00) = 0.77$ ,  $p = .441$ , GS1:  $b_{(CS-, GS1)} = 0.04$ ,  $SE = 0.04$ ,  $t(435.00) = 0.98$ ,  $p = .326$ , GS3:  $b_{(CS-, GS3)} = -0.01$ ,  $SE = 0.04$ ,  $t(435.00) = 0.21$ ,  $p = .838$ , GS4:  $b_{(CS-, GS4)} = -0.02$ ,  $SE = 0.04$ ,  $t(435.00) = 0.50$ ,  $p = .616$ ). Further trend analyses returned neither a Linear,  $F(1, 438) = 3.34$ ,  $p = .068$  nor a Quadratic trend across the test stimuli,  $F(1, 438) = 0.63$ ,  $p = .428$ . Mean signal topographies for ssVEPs in generalization can be found in [Figure 4](#).

#### 3.3.2 | Ratings

For valence, the linear mixed models yielded a significant main effect of Stimulus,  $F(5, 425) = 106.31$ ,  $p < .001$ ,  $R^2 = .556$ ,  $BF_{10} = 2.22e+68$ ,  $BF_{\text{inclusion}} = 1.48e+68$ , no difference for Group,  $F(2, 85) = 0.57$ ,  $p = .567$ ,  $R^2 = .013$ ,  $BF_{10} = 0.003$ ,  $BF_{\text{inclusion}} = 0.002$ , and a nearly significant Stimulus  $\times$  Group interaction,  $F(10, 425) = 1.76$ ,  $p = .066$ ,  $R^2 = .040$ ,  $BF_{10} = 1.38e+56$ ,  $BF_{\text{inclusion}} = 2.49e-12$ . Simple contrasts demonstrated that compared with CS-, participants found the CS+,  $b_{(CS-, CS+)} = 3.33$ ,  $SE = 0.18$ ,  $t(435.00) = 18.38$ ,  $p < .001$ , GS1,  $b_{(CS-, GS1)} = 2.31$ ,  $SE = 0.18$ ,  $t(435.00) = 12.73$ ,  $p < .001$ , GS2,  $b_{(CS-, GS2)} = 1.31$ ,  $SE = 0.18$ ,  $t(435.00) = 7.21$ ,  $p < .001$ , and GS3,  $b_{(CS-, GS3)} = 0.79$ ,  $SE = 0.18$ ,  $t(435.00) = 4.39$ ,  $p < .001$ , significantly more unpleasant

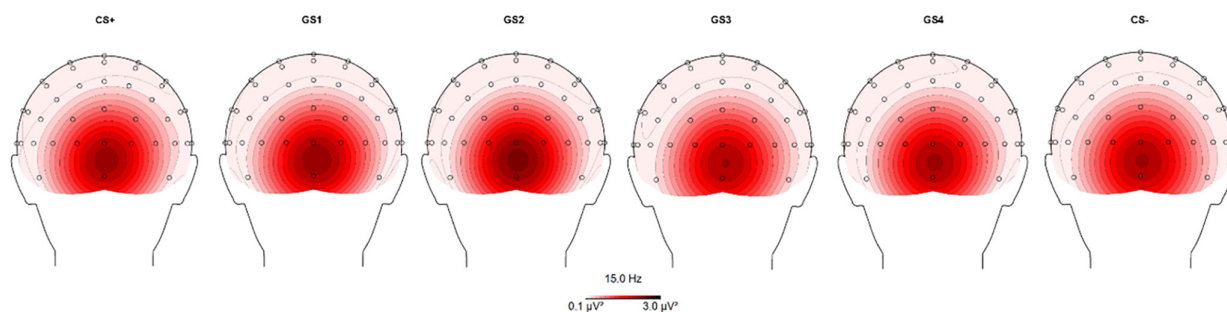


FIGURE 4 Mean scalp topographies of the 15 Hz signal power during generalization.

than the CS-, but not GS4,  $b_{(CS-, GS4)}=0.09$ ,  $SE=0.18$ ,  $t(435.00)=0.50$ ,  $p=.616$ . Trend analysis for valence ratings revealed significant Linear,  $F(1, 438)=494.11$ ,  $p<.001$  and Quadratic trends,  $F(1, 438)=24.79$ ,  $p<.001$ . Exploratorily, we found significant Linear trend  $\times$  Group interaction,  $F(2,434)=7.19$ ,  $p<.001$  but no Quadratic trend  $\times$  Group interaction,  $F(2,434)=0.36$ ,  $p=.695$ . All groups showed significant Linear trends, HU:  $F(1, 149)=213.98$ ,  $p<.001$ , LU:  $F(1, 129)=169.72$ ,  $p<.001$ , MU:  $F(1, 159)=110.19$ ,  $p<.001$ , but HU and MU had the biggest difference, and this might have caused the significant interaction. Figure 5 shows that the generalization responses for MU and HU look almost identical with lower responses for CS+ and higher responses for GS4 and CS- in MU.

Similarly, for arousal, there was a significant main effect for Stimulus,  $F(5, 425)=68.77$ ,  $p<.001$ ,  $R^2=.447$ ,  $BF_{10}=2.31e+47$ ,  $BF_{inclusion}=1.54e+47$ , but no main effect of Group,  $F(2,85)=0.10$ ,  $p=.902$ ,  $R^2=.002$ ,  $BF_{10}=0.002$ ,  $BF_{inclusion}=0.001$ , and a nearly significant Stimulus  $\times$  Group interaction,  $F(10, 425)=1.84$ ,  $p=.052$ ,  $R^2=.042$ ,

$BF_{10}=1.34e+35$ ,  $BF_{inclusion}=2.32e-12$ . Follow-up simple contrasts showed that compared with CS-, CS+,  $b_{(CS-, CS+)}=3.12$ ,  $SE=0.22$ ,  $t(435.00)=14.49$ ,  $p<.001$ , GS1,  $b_{(CS-, GS1)}=2.43$ ,  $SE=0.22$ ,  $t(435.00)=11.28$ ,  $p<.001$ , GS2,  $b_{(CS-, GS2)}=1.60$ ,  $SE=0.22$ ,  $t(435.00)=7.43$ ,  $p<.001$ , and GS3,  $b_{(CS-, GS3)}=0.81$ ,  $SE=0.22$ ,  $t(435.00)=3.74$ ,  $p<.001$  were found significantly more arousing, but there was no difference with GS4,  $b_{(CS-, GS4)}=0.25$ ,  $SE=0.22$ ,  $t(435.00)=1.16$ ,  $p=.247$ . Trend analysis for arousal revealed significant Linear,  $F(1, 438)=325.10$ ,  $p<.001$  and Quadratic trends,  $F(1, 438)=5.62$ ,  $p=.018$ . Again, we exploratorily followed up the nearly significant Stimulus  $\times$  Group interaction, and we found a significant interaction with the Linear trend  $\times$  Group,  $F(2, 434)=4.60$ ,  $p=.011$  with all groups showing a significant Linear trend, LU:  $F(1,129)=160.80$ ,  $p<.001$ , HU:  $F(1,149)=123.74$ ,  $p<.001$ , MU:  $F(1,159)=67.41$ ,  $p<.001$ . Similar to valence, CS+ responses in MU were lower than in HU and LU and slightly higher for GS4, which might have caused the nearly significant interaction. However, the Quadratic trend  $\times$  Group interaction was not significant,  $F(2, 434)=2.18$ ,  $p=.115$ .

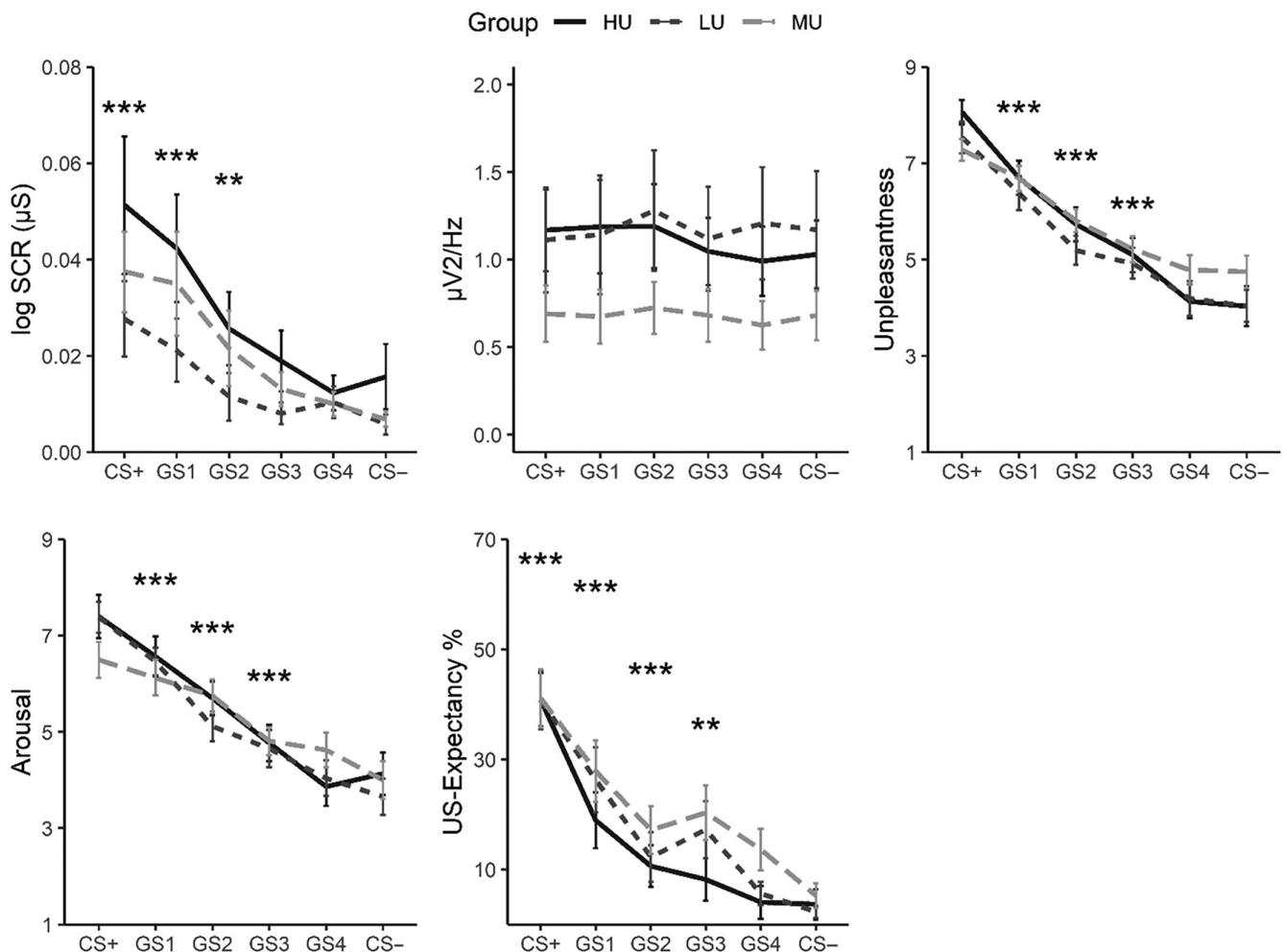


FIGURE 5 Means and standard error of the means for all test stimuli in the Generalization phase. Asterisks indicate significant difference from CS-.

Lastly, for US expectancy, there was a significant main effect of Stimulus,  $F(5, 425) = 62.16, p < .001, R^2 = .422, BF_{10} = 5.34e+44, BF_{inclusion} = 3.56e+44$ , but neither the main effect of Group,  $F(2,85) = 0.98, p = .379, R^2 = .023, BF_{10} = 0.005, BF_{inclusion} = 0.003$ , nor the Stimulus  $\times$  Group interaction reached significance,  $F(10, 425) = 0.91, p = .522, R^2 = .021, BF_{10} = 7.16e+30, BF_{inclusion} = 5.33e-14$ . Following the main effect of stimulus, simple contrasts with CS- as reference, revealed that participants expected significantly more threat after CS+:  $b_{(CS-, CS+)} = 37.09, SE = 2.39, t(435.00) = 15.46, p < .001, GS1: b_{(CS-, GS1)} = 20.52, SE = 2.39, t(435.00) = 8.56, p < .001, GS2: b_{(CS-, GS2)} = 9.61, SE = 2.39, t(435.00) = 4.01, p < .001, GS3: b_{(CS-, GS3)} = 11.42, SE = 2.39, t(435.00) = 4.76, p < .001$ , but not GS4:  $b_{(CS-, GS4)} = 4.14, SE = 2.49, t(435.00) = 1.72, p = .085$ . Trend analysis for US expectancy yielded both a Linear,  $F(1,434) = 261.96, p < .001$  and Quadratic trend,  $F(1,434) = 23.66, p < .001$ . US-expectancy ratings showed a steep, linear decrease from CS+ to GS2 and a less steep decrease from GS2 to CS-.

### 3.4 | Linear deviation score

The ANOVAs for the LDSs for the groups returned no significant results for SCR, ssVEPs, and valence (all  $p$  values  $> .345$ ). US expectancy and arousal approached significance,  $F(2,85) = 2.48, p = .090$  and  $F(2,85) = 2.87, p = .062$ , respectively. As shown in Table 4 participants in the MU group showed more generalization compared with HU in both US expectancy ( $p = .031$ ) and Arousal ( $p = .029$ ), but these differences did not survive Bonferroni correction ( $\alpha < .017$ ).

### 3.5 | Discrimination task

The three groups were additionally compared in the degree of discrimination of the CS+ from all other stimuli. The analysis returned no significant effect of Group,  $F(2,77) = 1.02, p = .367, \eta^2 = .025$ , so overall all groups discriminated well the CS+ from the other test stimuli, HU:

$M = 0.81, SD = 0.22, MU: M = 0.87, SD = 0.16, LU: M = 0.87, SD = 0.16$ .

## 3.6 | Exploratory analysis

### 3.6.1 | The relationship of intolerance of uncertainty and generalization (linear deviation score)

As an exploratory analysis, we correlated individual levels of IUS with generalization (LDS) in all variables included in the main analysis, to explore whether dispositional intolerance of uncertainty influences the steepness and strength of the generalization. We found a moderate positive correlation for US expectancy,  $r(86) = .324, p = .002$ . As can be seen in Figure 6, the higher the IUS of the participants, the wider the generalization gradient of their expectation of US in response to the GSs. IUS did not show significant correlations with any of the other measures (all  $p$  values  $> .208$ , Bonferroni correction  $\alpha < .01$ ).

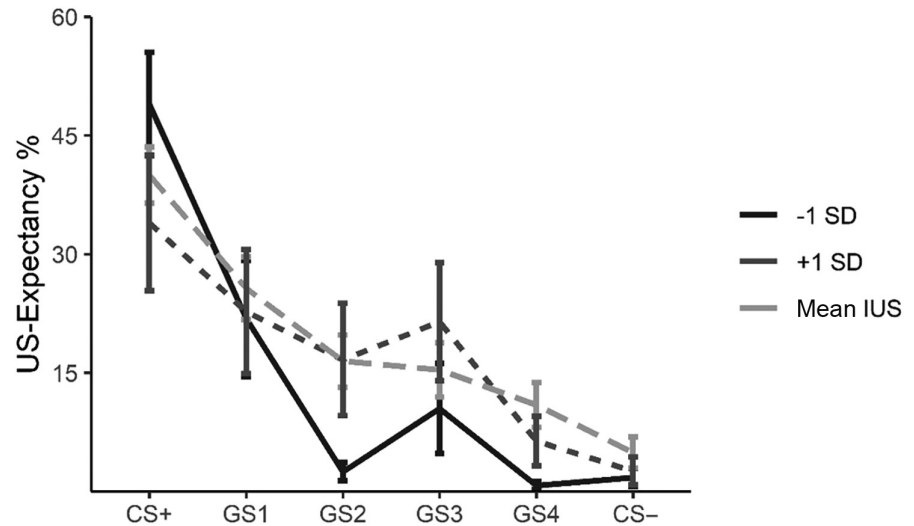
## 4 | DISCUSSION

In this study, we explored whether threat uncertainty, expressed in different reinforcement schedules between CS+ and US, could lead to wider fear generalization. A second aim was to see whether fear generalization would be differentially expressed in various systems involved such as showing lateral inhibition in the visual cortex but linear generalization in autonomic arousal and subjective ratings. In contrast to our expectations, threat uncertainty did not lead to overgeneralization of the threat responses in any of the measured variables. These findings are partly in agreement with Zhao et al. (2022), who found no evidence for influence of the reinforcement rate on autonomic arousal in generalization but, contrary to our findings, found increased threat expectancy ratings for the partial reinforcement groups. However, in both studies, threat uncertainty was not associated with wider generalization gradients.

**TABLE 4** Means and standard deviations of the groups for the linear deviation scores in the different measures.

Variable	HU	MU	LU	Total
	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>	<i>M (SD)</i>
US-exp (0–100)	-11.81 (14.30)	-3.45 (15.37)	-6.23 (15.21)	-7.12 (15.21)
Arousal (1–9)	-0.54 (1.29)	0.08 (1.07)	-0.45 (0.86)	-0.29 (1.12)
Valence (1–9)	-0.63 (1.15)	-0.39 (1.05)	-0.62 (0.94)	-0.54 (1.05)
SCR (microS)	-0.01 (0.02)	-0.02 (0.01)	-0.00 (0.01)	-0.00 (0.02)
ssVEPs (Power FFT)	-0.01 (0.33)	-0.02 (0.22)	0.09 (0.24)	0.01 (0.27)

**FIGURE 6** Means and standard error of the means for all test stimuli in US expectancy for each level of IUS scores in the Generalization phase.



One reason for the absence of differential generalization gradients between groups in our study could be successful acquisition of conditioned fear in all groups. In line with previous studies, participants found CS+ more arousing, unpleasant, more likely to be followed by the US, and more physiologically arousing compared to CS- (Ahrens et al., 2016; Dunsmoor et al., 2017; Herzog et al., 2021; Lemmens et al., 2021; McClay et al., 2020; Stegmann et al., 2020). Impaired discriminative fear learning is often found in people with anxiety and stressor-related disorders (Cha et al., 2014; Greenberg et al., 2013; Huggins et al., 2021; Lissek et al., 2009, 2010, 2014; Milad et al., 2007) and is hypothesized to carry over into the generalization phase leading to less steep (i.e., more linear) generalization gradients. The clear discrimination between threat and safety cues in our study could have minimized the manifestation of overgeneralization despite the fact that threat uncertainty differed across groups (Lenaert et al., 2014). Another reason could be that threat uncertainty expressed in different reinforcement schedules is not strong enough to lead to overgeneralization. Despite the fact that the uncertainty manipulation was reflected in threat expectations and autonomic arousal during acquisition, it did not modulate the affective ratings. Additionally, the various phases of a threat conditioning paradigm involve different levels of uncertainty (Morriss et al., 2021). For example, acquisition includes uncertainty concerning the CSs, the contingencies, and their reinforcement. This uncertainty decreases as the experiment proceeds and the participants acquire more information regarding these aspects. Generalization includes inherently a greater degree of uncertainty as, in addition to the factors mentioned earlier, new stimuli are introduced (the GSs) and their contingencies to the US are unknown. In our study, all groups received the same reinforcement during generalization, and thus, both the inherent uncertainty of the generalization phase and the one caused by the reinforcement

rates were the same. It is therefore conceivable that threat uncertainty as a result of the reinforcement rate during acquisition was not strong enough to override the uncertainty in the generalization phase, and that is why all groups showed similar generalization gradients. However, it is possible to isolate the influence of the reinforcement rates if these are different for the groups in the generalization phase as well.

Although fear generalization was not modulated by the manipulation of threat uncertainty, we found that higher trait intolerance of uncertainty was associated with wider generalization in threat expectancy ratings. The impact of intolerance of uncertainty in fear generalization is still somewhat unclear. Results from studies so far point to less discrimination of SCR responses to the CSs and GSs in acquisition for people with high intolerance of uncertainty; however, this finding is inconsistent (Bauer et al., 2020; Morriss et al., 2016; Nelson et al., 2015) and so far there was no correlation with fear generalization (Mertens et al., 2021). In the current study, we found a moderate correlation with threat expectancy ratings. A difference with the previous studies described (Bauer et al., 2020; Morriss et al., 2016; Nelson et al., 2015) is that the acquisition phase in the current study did not include any GSs, and thus, in the generalization phase, participants saw these stimuli for the first time. From studies so far, including the current study, it is clear that partial reinforcement induces uncertainty and it is a good method to demonstrate the role that intolerance of uncertainty plays in fear generalization since all these studies use partial reinforcement, but no such evidence has been found with higher reinforcement schedules (75%; Mertens et al., 2021). Additionally, since the US-expectancy ratings in this study were retrospective, we measured the overall subjective feeling of threat expectancy participants had at the end of the experiment. Our findings show that partial reinforcement can influence the generalized responses of a subset

of participants scoring high in intolerance of uncertainty, and therefore, the reinforcement schedule should be carefully considered in fear generalization studies. Since this analysis was of exploratory nature, it should be considered with caution, and further research would be needed to clarify the role of trait intolerance of uncertainty on the different facets of fear generalization.

Our second aim was to examine whether fear generalization would show different responses in the various systems involved. No such differences were observed in the generalization phase; however, our results demonstrate different mechanisms involved in fear learning between threat expectancy, autonomic arousal, and affective ratings. More specifically, although participants expected less threat in the high-uncertainty group, they displayed higher autonomic arousal compared with the low-uncertainty group. This finding adds to existing literature demonstrating higher SCR with higher uncertainty (de Berker et al., 2016; Tzovara et al., 2018) as well as unpredictability of threat (Alvarez et al., 2015; Dretsch et al., 2016). However, not all studies found modulation of SCR by uncertainty induced by the reinforcement rate (Zhao et al., 2022). This difference could be because in the study by Zhao et al., participants might not have been aware of the reinforcement as the three groups did not display significant differences in threat expectancy either. However, uncertainty about future events and threats increases the affective reactions to these events (Bar-Anan et al., 2009; Grillon et al., 2004, 2008). Therefore, our results suggest that increased uncertainty is linked to increased autonomic arousal despite low probability of threat and could therefore reflect the effort to successfully predict the threat.

It is worth mentioning that threat uncertainty in our study was not enough to differentiate the groups in the affective ratings. On the one hand, one would expect that low threat expectancy will not cause very unpleasant and arousing feelings. However, our findings show that the CS+ was equally unpleasant and arousing regardless of low expectation of the threat. This pattern resembles the difficulty people with clinical anxiety have suppressing their defensive reactions despite concrete knowledge that these reactions are exaggerated. On the other hand, expectancy and affective learning are thought to represent distinct learning processes that can take place during classical conditioning (Hamm & Vaitl, 1996; Hamm & Weike, 2005; Hermans et al., 2002; Lonsdorf et al., 2017). Expectancy-learning refers to the association that the CS activates the expectation of the US in the immediate future, and it is associated with measures that relate to conscious awareness such as SCR and US expectancy (e.g., (Biferno & Dawson, 1977; Dawson & Biferno, 1973; Ross & Nelson, 1973). Affective learning refers to the process by which CS presentation activates the representation of the US and its positive/negative valence

without activating its expectation. Additionally, whereas expectancy learning seems to be related to more conscious defensive processes such as SCR, affective learning is related to more unconscious processes such as fear-potentiated startle responses (Bradley & Lang, 1994; Hamm & Vaitl, 1996; Hamm & Weike, 2005). Our findings are in line with this distinction between affective and cognitive learning mechanisms as our manipulation mainly focused on the expectancy and not necessarily on the valence or arousal of threat. In turn participants' threat expectations and autonomic arousal were affected by threat uncertainty, whereas valence and arousal perceptions remained unaffected.

Contrary to our expectations and previous literature (Keil et al., 2013; McTeague et al., 2015; Miskovic & Keil, 2013; Petro et al., 2017; Stegmann et al., 2020), we found no differential responding in the visual cortex, neither in the acquisition nor in the generalization phase. A closer look in the literature revealed several factors that could explain the absence of discriminatory visuocortical responding. First, the majority of the previous studies (Gruss & Keil, 2019; McTeague et al., 2015; Miskovic & Keil, 2013; Moratti & Keil, 2005) used basic perceptual CSs such as Gabor gratings of different orientations. Such simple stimuli can directly engage orientation-sensitive cells in the visual cortex (Hubel & Wiesel, 1962), and therefore, the differential processing of CS+ related orientations compared with the ones related to CS- is easier to detect with EEG. However, such differential engagement can be difficult to detect using complex stimuli such as faces that include multiple features. In complex stimuli, threat-related features could still be selectively enhanced, but this difference is more difficult to be detected because the stimuli might share more similarities than differences (McTeague et al., 2015). Another reason can be the viewing distance of the stimuli. In previous studies using ssVEPs (Gruss & Keil, 2019) and complex stimuli such as the ones used in the current study (Kastner-Dorn et al., 2018; Stegmann et al., 2020; Wieser et al., 2014), participants were sitting 100 cm away from the screen, whereas in our study, they were sitting 150 cm away. Stimuli presented with greater perceived distance have smaller angular size and smaller cortical representation (Murray et al., 2006). Thus, the combination of complex stimuli, such as faces, and the longer distance of the stimuli might have influenced the visuocortical engagement and made the differences too small to detect. Furthermore, a closer review of the literature revealed that the differential CS cortical engagement is not consistently reported with ssVEPs (Friedl & Keil, 2020) and often depends on other individual characteristics such as genotype (Gruss et al., 2016) and heart rate (Moratti et al., 2006; Moratti & Keil, 2005), which were not included in this study. The inconsistent results warrant the need for a systematic review of the available studies to determine the consistency and size of the effect.

This study has several strengths and some limitations. First, the examination of psychophysiological, cognitive, and affective measures allows us to follow fear generalization from the very first moments of threat perception and track how it is manifested in the brain, body, cognitive, and affective processes. Although we could not observe discriminatory responses in visuocortical responding, further exploration is needed to examine the size of the effect and how it can be better studied or explore other methods that could capture early stages of fear generalization in the brain such as the late positive potential (LPP; Nelson et al., 2015). Second, in contrast to Zhao et al. (2022) where the generalization's reinforcement rate was identical to acquisition for one of the groups, we kept the reinforcement rate of the generalization phase at 20% that was lower than the acquisition phase but comparable across the groups. Regarding the limitations, the duration of the experiment was fairly long, which could have influenced the SCR. Since no instructions were given to the participants about the reinforcement schedule, we needed to ensure that enough learning trials would be available. This resulted in a duration of 45 mins, which could have induced a strong habituation of the psychophysiological responses during the generalization phase (Codispoti et al., 2006; Peeke, 2012) and could have constituted potential differences between the stimuli too small to detect. Second, in the generalization phase, all groups had the same reinforcement rate of 20% to ensure that the test phase for the generalization processes was comparable across groups. However, this resulted in an asymmetrical decrease in reinforcement from acquisition to generalization across groups. More specifically, the CS-US contingency in LU was reduced by 75%, in MU by 66%, whereas in the HU group by 50%. The asymmetrical decrease from acquisition to generalization could have led to earlier extinction in LU and an artificial difference between the groups. However, this did not constitute a problem in our study as no differences were observed among the groups. Furthermore, there were no strong differences between the group with moderate uncertainty in comparison with the other two already in the acquisition, suggesting that the difference between the reinforcement schedules chosen in this study might have been too small for participants to detect (80%, 60%, and 40%). The difference in the reinforcement rates between the groups chosen by Zhao et al. (2022) was slightly bigger (25%) but resulted in even smaller differences. Therefore, a larger difference in reinforcement rate, but also more trials may lead to larger group differences in generalization in future studies. Additionally, we did not ask our participants how "uncertain" they felt while seeing the visual stimuli during the experiment. Uncertainty can be seen both as an external and an internal condition (Grupe & Nitschke, 2013). We explicitly manipulated external uncertainty, but a subjective (or

internal) uncertainty could have additionally influenced participants' responses and may be especially interesting for anxiety psychopathology. Finally, although we have no information regarding the ethnicity of the actresses in the pictures included in this study, using only White, female faces limits the generalizability of our results.

To conclude, our study successfully replicated fear acquisition and fear generalization on both verbal and physiological responses. Participants clearly distinguished between threat and safety signals and generalized their fear only to those stimuli similar to the threat signal. The reinforcement schedule and therefore the uncertainty of the threat did not influence the generalization gradient of the three learning groups, but higher intolerance of uncertainty was associated with wider expectancy of threat in generalization. Interestingly, we found different responses in the subjective ratings by the uncertainty reflected in the reinforcement rate as this was observed in participants' US-expectancy ratings, but not in the valence and arousal ratings. Finally, our results support the notion that lower predictability and therefore higher uncertainty of threat leads to increased autonomic arousal.

#### AUTHOR CONTRIBUTIONS

**Asimina Aslanidou:** Conceptualization; data curation; formal analysis; investigation; methodology; writing – original draft. **Marta Andreatta:** Formal analysis; methodology; writing – review and editing. **Alex Wong:** Formal analysis; writing – review and editing. **Matthias Wieser:** Conceptualization; formal analysis; methodology; supervision; writing – review and editing.

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#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Open Science Framework at <https://doi.org/10.17605/OSF.IO/TDQJ3>.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**TABLE S1.** Information regarding the ethnicity of the participants included in the study.

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