

Contingency Reversal in Conditioned Fear Learning: The Moderated Mediation Model of Intolerance of Uncertainty and Instruction

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Objective: The study aimed to examine the roles of anxiety and intolerance of uncertainty (IU) in conditioned fear learning under an uncertain context induced by the contingency reversal of the association between the conditioned stimulus and the unconditioned stimulus (CS-US).

Methods: The study sample comprised 53 participants, randomly divided into two groups: a non-instruction group and an instruction group. The experimental procedure encompassed five stages: pre-acquisition, acquisition, generalization, reversal acquisition, and reversal generalization. Our study primarily focused on analyzing a moderated mediation model.

Results: In the instructed group, we observed that the reversed fear generalization response was directly influenced by the pre-reversal fear generalization response, while also being indirectly mediated by the IU factor. However, in the non-instructed group, we did not find a significant mediating effect of IU. Moreover, we noted that the mediation of IU was contingent on the instructional information. It is noteworthy that anxiety did not exhibit a discernible role in conditioned fear within the uncertainty condition in our study.

Conclusion: The findings provide novel insights into fear-related phenomena, emphasizing the intricate interplay between individual traits and fear generalization under conditions of uncertainty. They contribute to understanding the mechanisms of emotional and cognitive interactions in uncertain conditions.

Keywords: associations learning, fear generalization, instructional learning, moderated mediation model

Introduction

Fear represents a potent emotional sensation often linked with scenarios involving threat, peril, or a sense of insecurity.¹ It embodies an innate physiological and psychological reaction, motivating individuals to undertake appropriate actions for self-preservation or managing potential threats. Fear serves a protective function in evolution, aiding us in identifying potential dangers and instigating appropriate self-defense or avoidance behaviors.^{2,3}

In the context of fear conditioning, individuals acquire fear of conditioned stimuli (CS), resulting in conditioned fear responses. However, an increasing corpus of empirical evidence suggests that fear responses are not exclusively elicited by the original CS. Rather, stimuli sharing features with these original stimuli also have the capacity to evoke fear responses.⁴ This phenomenon is denoted as fear generalization, and non-original stimuli capable of inducing fear are termed generalization stimuli (GS).⁵ A justifiable fear generalization response can be seen as a mechanism of self-preservation. It enables individuals to heighten their vigilance and respond appropriately in the face of potential threats. By extending fear responses to analogous contexts or stimuli, individuals can better predict and counteract potential dangers, bolstering their survival and safety even when specific threats are not present. However, fear generalization responses can also arise through irrational or excessive influences, leading individuals to avoid situations or stimuli-linked objects, and limiting their exploration of the environment.⁶⁻⁸ Individuals might refrain from certain activities or

steer clear of particular locales to circumvent these fear responses. Excessive fear generalization can profoundly impact an individual's overall well-being, and may give rise to sustained fear and anxiety,^{9,10} associated with psychological and emotional turmoil. Joy in daily activities may even diminish and be replaced with an enduring sense of tension and disquietude.

Experimental research indicates that fear generalization is commonly observed when there is perceptual similarity between CS and GS, alignment of CS and GS within categorical or conceptual categories, or when CS and GS share analogous abstract rules.¹⁰ Perceptual similarity refers to the presence of sensory resemblance between the CS and GS, such as shared external stimulus features including shape, color or odor.^{5,11,12} Conceptual category relevance involves the association of CS and GS on a conceptual level, signifying their semantic relatedness.^{13–15} When CS and GS share similar concepts or belong to the same category, the likelihood of fear generalization increases. Rule consistency refers to the phenomenon where individuals, having learned the conditioned stimulus and unconditioned stimulus (CS-US) association based on specific rules, and extend fear responses during the process of recognizing GS that possess analogous rules.¹⁶ Indeed, fear generalization can only occur subsequent to the acquisition of CS-US associations. Therefore, when assessing the potential threat association of GS, individuals tend to draw on their previous learning experiences. They are inclined to rely on specific attributes of the CS, and the established CS-US association, to infer the level of threat posed by GS. At this point, fear generalization hinges on the similarity or relevance between GS and CS along a specific dimension. More shared characteristics between GS and CS lead to more robust generalization responses. This process describes the generalization of both threat and safety responses.¹⁷

In fear generalization testing, GS emerge as entirely untrained neutral stimuli in comparison with CS. Here, GS exhibit novel distinctiveness and inherent uncertainty distinct from CS. In potential threat scenarios, humans tend to associate uncertainty with threat and anticipate negative outcomes.¹⁸ Driven by evolutionary and adaptive significance, individuals are prone to excessively identifying cues of threat or danger in potential threat situations. This tendency involves perceiving non-threatening stimuli or contexts as threatening and eliciting corresponding fear responses. The scenario is similar to signal detection theory and the situation of false positives where a non-signal (noise) is erroneously identified as a signal. This results in the activation of the fear-based vigilance system, aimed at ensuring the detection of potential threats. In this context, individuals tend to set lower decision thresholds, thereby enhancing sensitivity to potential threats (manifesting as fear expectations toward GS).² The behavior may be interpreted as an excessively self-protective response, aimed at avoiding the potential oversight of real threats.¹⁹ However, this self-regulation to adapt to uncertainty is not always effective. Chronic stress results in adverse effects when uncertainty remains unresolved or is resolved too late.²⁰

Grupe and Nitschke¹⁸ found that participants tended to link uncertain expectations with aversive outcomes. Aversive outcomes linked to uncertainty cues elicited a heightened reporting of negative emotions in contrast with aversive outcomes linked to certain cues. This highlights how uncertainty magnifies the negative effects of aversive events, resulting in individuals excessively linking uncertainty with adverse outcomes.²¹ More robust evidence emerges from experiments conducted during fear generalization tests, where it is often observed that when the GS is repeatedly unpaired with the US, individuals tend to develop a newly acquired sense of safety associated with the GS. This manifests in a progressive decline in fear expectancy toward the US linked to the GS as the trials unfold.²² These findings underscore the notion that as the GS loses its novelty, the uncertainty surrounding the GS-US association diminishes, leading to reduced generalization.

In the context of conditioned fear, uncertainty can be introduced by varying the reinforcement rates during the training of the CS and US.²³ Zhao et al²⁴ employed a partial reinforcement paradigm, introducing uncertainty factors in the pairing of CS and US. The research revealed consistent levels of fear acquisition in both continuous reinforcement (100%) and partial reinforcement conditions (75%, 50%). Notably, under partial reinforcement conditions, a more pronounced fear generalization effect manifested. Moreover, uncertainty can be introduced through the reversal of the CS-US association.^{25,26} Studies on the reversal of threat contingencies demonstrated a generalization-enhancing effect, with contingency reversal leading to sustained increases in fear-potentiated startle and heightened perceived risk.²⁷ Due to the presence of uncertainty, fear learning becomes intricately complex and flexible, requiring individuals to delve

deeper into the relationship between stimuli and fear responses. This increased attentiveness promotes a heightened sensitivity to changes in conditioning,²⁰ potentially contributing to a more pronounced fear generalization.

Individual traits or variances in intolerance of uncertainty (IU) and anxiety should be taken into account when exploring the extent to which uncertainty contributes to fear generalization responses. These factors may play a significant role in the process of conditioned fear generalization. Maladaptive fear generalization reflects aberrations in the regulation and processing mechanisms of fear and anxiety. The prevailing perspective posits that maladaptive fear generalization is a pathogenic marker of clinical anxiety.^{28–30} Moreover, this maladaptive process is recognized as a shared characteristic in several anxiety disorders, such as panic disorder and generalized anxiety disorder.²⁸ Excessive fear generalization has been associated with high trait anxiety, elevated state anxiety, and heightened intolerance to uncertainty.^{29,31,32} In clinical settings, patients with anxiety-related disorders exhibit higher degrees of fear generalization compared with healthy individuals.^{33,34} Among non-clinical samples, individuals with elevated anxiety traits exhibit diminished capacity for distinguishing between threats and safe cues, alongside impaired safety learning and generalization.^{35,36} In potential threat conditions, anxiety is characterized by the tendency to perceive neutral stimuli as unsafe, and anxious individuals are more inclined than non-anxious individuals to construe ambiguous scenarios as posing threats.

Furthermore, individuals diagnosed with social anxiety disorder, generalized anxiety disorder, obsessive-compulsive disorder, and major depressive disorder often report similar degrees of IU. This implies that IU may represent a prevalent vulnerability factor across most anxiety disorders.³⁷ Individuals with high IU exhibit heightened sensitivity to uncertainty, often experiencing a diminished sense of control over unfamiliar situations or contexts. Consequently, they are prone to experiencing heightened levels of anxiety, tension, and apprehension related to uncertainty.³⁸ Research focused on the relationship between IU and fear generalization has demonstrated that individuals with high IU struggle to discriminate between safe cues and threat cues.³⁹ This difficulty leads to compromised learning of safety associations, resulting in a sustained perception of threat toward both safe and neutral stimuli.^{2,40} These individuals also exhibit heightened anticipation of threat regarding GS. As a consequence, they encounter challenges in acquiring cues of safety,⁴¹ and face challenges in the process of fear extinction and fear reduction.⁴⁰ Hence, it is crucial to study individual differences in anxiety and IU traits among individuals, and their distinct manifestations in fear generalization. This focus will promote a deeper comprehension of the mechanisms underlying fear generalization and facilitate accurate identification and support of individuals who exhibit excessive fear generalization.

However, there is limited research on the influence of the intrinsic uncertainty inherent in GS on fear generalization. This gap makes it challenging to ascertain how fear generalization occurs and how it generates differential responses among individuals. The present study aimed to investigate the role of uncertainty in fear generalization by employing a reversal procedure that alters CS-US associations.^{26,42,43} By inducing uncertainty regarding the relationship between stimuli and threat outcomes, we sought to compare the initial fear generalization response before the reversal with the changes observed in fear generalization response after the reversal. The experiment incorporated two distinct groups: an instruction group exposed to reversal information and a non-instruction group not provided with such information.^{44–46} This configuration was employed to examine the disparities in fear generalization responses under two distinct uncertainty conditions, aiming to delve into the fear generalization process in contexts with varying uncertainty. We prompted participants to acquire dual threat cues and one safe cue, followed by a manipulation in which one of the threat CS was transformed into a safe cue during the reversal stage.

The advantage of the research design lies in its ability to comprehensively achieve the research objectives and effectively prevent the potential acquisition of safety responses to the GS stimuli based on the similarity between pre- and post-reversal generalization testing procedures, or rather, the attenuation of potential fear expectancies caused by repeated exposure to the GS without US. More importantly, the retention of the pre-reversal fear generalization testing procedure allowed us to establish a baseline level of fear expectancy responses during initial generalization. This measure addressed the potential influence of fear generalization responses associated with stimuli similarity, and enriched the availability of information about individual differences, enabling a robust interpretation of the results from the post-reversal generalization testing stage.

Methods and Participants

Participants

Volunteers were recruited for the experiment and randomly allocated to one of the two groups. They were between the ages of 18 and 35 and had to have normal vision, no color blindness, no history of mental health difficulties, and no history of taking psychotropic drugs. Additionally, they should not have participated in any similar shock-related experiments within the last six months. A total of 57 participants took part in the study. Four participants were excluded because of failure to acquire CS-US associations after the acquisition stage. Data were collected from 53 participants, with an average age of 21.02 (standard deviation (*SD*) = 2.693). Participants were informed of the possibility of receiving electric shocks during the experiment and signed an informed consent form before the start of the experiment. All participants were compensated at the end of the study. This study was conducted in compliance with the principles of the Declaration of Helsinki and received approval from the Ethics Committee of the School of Psychology, South China Normal University, with the approval number: SCNU-PSY-2022-179.

In the laboratory, participants were seated approximately 60 centimeters away from the monitor and connected to the equipment. Prior to the formal experiment, they completed the State-Trait Anxiety Inventory⁴⁷ to measure state and trait anxiety levels, the Cronbach's α values were 0.894 and 0.866. Additionally, the Intolerance of Uncertainty-Short Form⁴⁸ was administered to measure IU, Cronbach's α value was 0.830. Then their shock tolerance was measured. Experimenters manually operated the stimulator to deliver 200 ms shocks, and participants were asked to verbally rate the intensity on a scale of 1 (no sensation) to 9 (extremely uncomfortable, unbearable). The shock intensity that participants rated as 8 (extremely uncomfortable but tolerable) was used as the US throughout the experiment. The shock intensities in this study ranged from 35 V to 87 V, with a mean of 57.32 V (*SD* = 11.961). Two participants reported that the shocks were too intense during the experiment and requested a reduction in shock intensity (from 66 V to 50 V and from 58 V to 50 V). The data from these two participants were included in the analysis. At the start of the task, participants were instructed to focus on the visual stimuli presented on the screen and to predict the shock associated with the shapes. Within a 5-second window, participants were required to respond with a numeric keypress ranging from 1 to 9, indicating the expectancy rating of receiving a shock (where 1 = impossible and 9 = very likely).

Materials

The visual stimuli used in this study were green shape images displayed on a black background with dimensions of 300×300 pixels (see Figure 1A). There were three CS stimuli: CS threat (CSt), CS reversal (CSr), and CS safe (CSs). The six shapes that transitioned continuously between CSt (circle) and CSs (square) were labeled GS1 to GS6, and the six shapes that transitioned continuously between CSr (triangle) and CSs (square) were labeled NGS1 to NGS6. We ensured a balanced presentation of the CS images during the experiment.

The positive and negative terminals of the Digitimer DS2A isolated voltage stimulator (Hertfordshire, UK) were connected to participants' right wrists with disposable electrodes, and the apparatus produced 200 ms of electrical stimulation in the experiment.

Procedures

The formal experiment consisted of five stages: pre-acquisition, acquisition, generalization, reversal acquisition, and reversal generalization. The pre-acquisition stage involved two trials each of CSt, CSr, and CSs without electric shocks. The acquisition stage presented six trials each of CSt, CSr, and CSs. Of these, shocks were applied to CSt and CSr in four out of six trials (with the US reinforcement rate of 75%), while CSs were never paired with shock. During the generalization stage, participants were presented with three trials each of CSt (CS+), GS1–GS6, and CSs (CS-) in total. CSt stimuli maintained a 75% shock reinforcement rate, with two trials paired with shocks, while other stimuli were presented without US. In the reversal acquisition stage, CSs were presented in six trials, with four trials paired with US, resulting in a new CS+ (NCS+), whereas CSr was presented in six trials without US, resulting in a new CS- (NCS-). The non-instruction group did not receive any information about the reversal of CS-US associations, while the instruction group received instructions about the reversal of CS-US associations. During the reversal generalization stage,

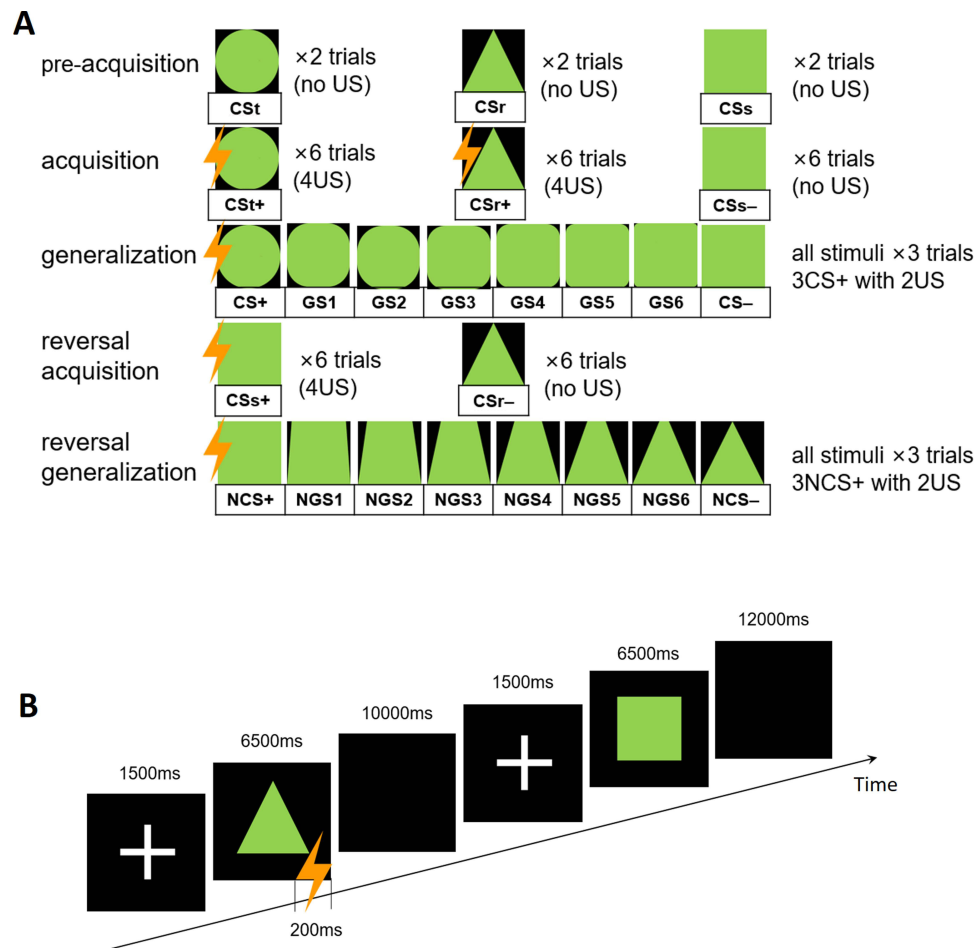


Figure 1 The experiment procedure.

Notes: (A) The five experimental stages along with the complete set of stimuli employed in the experiment, encompassing three conditioned stimuli (CS), including CS threat (CS_t), CS reversal (CS_r) and CS safe (CS_s), and the six generalization stimuli (GS) and six new GSs (NGS), US refers to the unconditioned stimulus. (B) The schematic representation of stimuli presentation during the experiment.

participants were presented with three trials each of CSs (NCS_t), NGS1–NGS6, and CS_r (NCS_s) in total. Shocks were administered during two of the NCS_t trials. The entire experiment lasted approximately 40 minutes.

For the initial stage of each trial, a white cross was displayed at the center of a black screen for 1500 ms. This was followed by a visual stimulus display that lasted for 6500 ms. During the presentation of the visual stimulus, a scoring bar emerged at the bottom of the screen and participants were advised to use the numeric keypad to express their expectancy of the impending shock (1 for less likely, 9 for very likely). The shock followed the trials at 6300 ms and lasted for 200 ms, coinciding with the end of the presentation of the shape. It was followed by the appearance of a black screen. The duration of the black screen varied between 10,000 and 12,000 ms (see Figure 1B).

Analysis

We analyzed participants' fear expectancy ratings toward the stimuli using SPSS 24.0 and R 4.2.1 during the pre-acquisition, acquisition, and reversal acquisition stages. Mixed ANOVAs were used to assess learning performance across the different conditions and identify disparities in fear responses between the acquisition and reversal acquisition stages. We performed a mediation analysis using Mplus 7.0 to comprehensively investigate differences in fear generalization responses before and after the reversal, across individuals with varying levels of anxiety and IU within the two groups. To bolster the robustness of our analysis, especially considering the limited sample size, we incorporated data from each participant's three trial responses to every stimulus during both the generalization and reversal generalization

stages. Furthermore, we explored the possibility of differences in mediation effects among the two participant groups. Statistical significance was set at $p < 0.05$.

Results

There were no significant gender ratio, age distribution, shock tolerance, state anxiety, trait anxiety, or IU differences between the two groups. Similarly, no significant differences were observed between the two groups in their fear expectancies toward the three stimuli (CSt, CSr, and CSs) during the pre-acquisition stage (Table 1).

Fear Acquisition

During the pre-acquisition stage, we calculated the mean fear expectancies for the two trials of CSt, CSr, and CSs. No significant differences were found in fear expectancies for the three stimuli, with $F(2, 102) = 0.941$, $p = 0.394$, $\eta_p^2 = 0.018$. Prior to formal acquisition, the fear responses to these stimuli were equivalent among the participants, and no interaction between the stimulus type and group, with $F(2, 102) = 1.178$, $p = 0.312$, $\eta_p^2 = 0.023$ (see Figure 2). This indicates that before commencing the acquisition training, both groups had an equivalent level of fear expectancy toward these three stimuli.

Significant differences were found in US expectancy rating among the three stimuli during the fear acquisition stage, $F(2, 102) = 70.749$, $p < 0.001$, $\eta_p^2 = 0.581$, 95% CI [0.451, 0.664]. CSt had a mean US expectancy of 6.380 ($SD = 1.238$), which was significantly higher than the mean US expectancy of 3.678 ($SD = 1.578$) for CSs, $p < 0.001$, $MD = 2.702$, $SE = 0.279$, 95% CI [2.171, 3.233]. There was also a significant difference in US expectancy observed between CSr vs. CSs, $p < 0.001$, $MD = 2.562$, $SE = 0.280$, 95% CI [2.001, 3.124], indicating that participants successfully discriminated between the two threat stimuli and one safety stimulus during acquisition. There was no significant difference in US expectancy between CSt and CSr, $p = 0.526$, $MD = 0.140$, $SE = 0.219$, 95% CI [-0.300, 0.580], indicating that participants acquired an equivalent level of threat valence for the two threat stimuli. Moreover, there was no significant interaction effect between group and CS, $F(2, 102) = 0.191$, $p = 0.872$, $\eta_p^2 = 0.004$. Overall, both groups exhibited consistent conditioned fear during the acquisition stage.

We observed a significant three-way interaction of trial $\times$ group $\times$ CS during the reversal acquisition stage, $F(5, 255) = 9.095$, $p < 0.001$, $\eta_p^2 = 0.151$, 95% CI [0.067, 0.218]. Further simple effects analyses revealed that the instruction group could discriminate the NCS+ and NCS- as early as trial 1, $p < 0.001$, $MD = 2.259$, 95% CI [1.217, 3.302]. In contrast, the non-instruction group required until trial 2 to establish the new association, $p = 0.039$, $MD = 1.385$, 95% CI [0.073, 2.696]. The non-instruction group, devoid of knowledge regarding the CS-US reversal, exhibited a delayed acquisition of

Table 1 Overview of the Characteristics of the Two Participant Groups

	Non-Introduction Group (M±SD)	Introduction Group (M±SD)	Comparison		
			χ^2	t	p
Gender (male/female)	7/19	8/19	0.048		0.872
Age (year)	20.92±1.787	21.11±3.378		-0.252	0.802
Shock (volt)	60.54±13.356	54.22±9.710		1.975	0.054
STATE ANXIETY	38.62±9.567	38.48±9.263		0.052	0.959
TRAIT ANXIETY	39.69±9.311	39.44±6.441		0.112	0.911
IU	27.73±5.583	26.52±6.129		0.752	0.456
CS threat (CSt)	4.11±2.246	4.03±1.743		0.149	0.882
CS reversal (CSr)	4.13±2.520	4.83±1.709		-1.215	0.230
CS safe (CSs)	4.01±1.713	4.81±1.954		-1.584	0.119

Notes: the table includes information related to gender ratio, age distribution, shock tolerance, state anxiety, trait anxiety, and intolerance of uncertainty. The table also presents data on fear expectancies for the three stimuli during the pre-acquisition stage. No significant differences were observed between the two groups across these characteristics, indicating their comparability for the study.

Abbreviations: IU, intolerance of uncertainty; CS, conditioned stimulus.

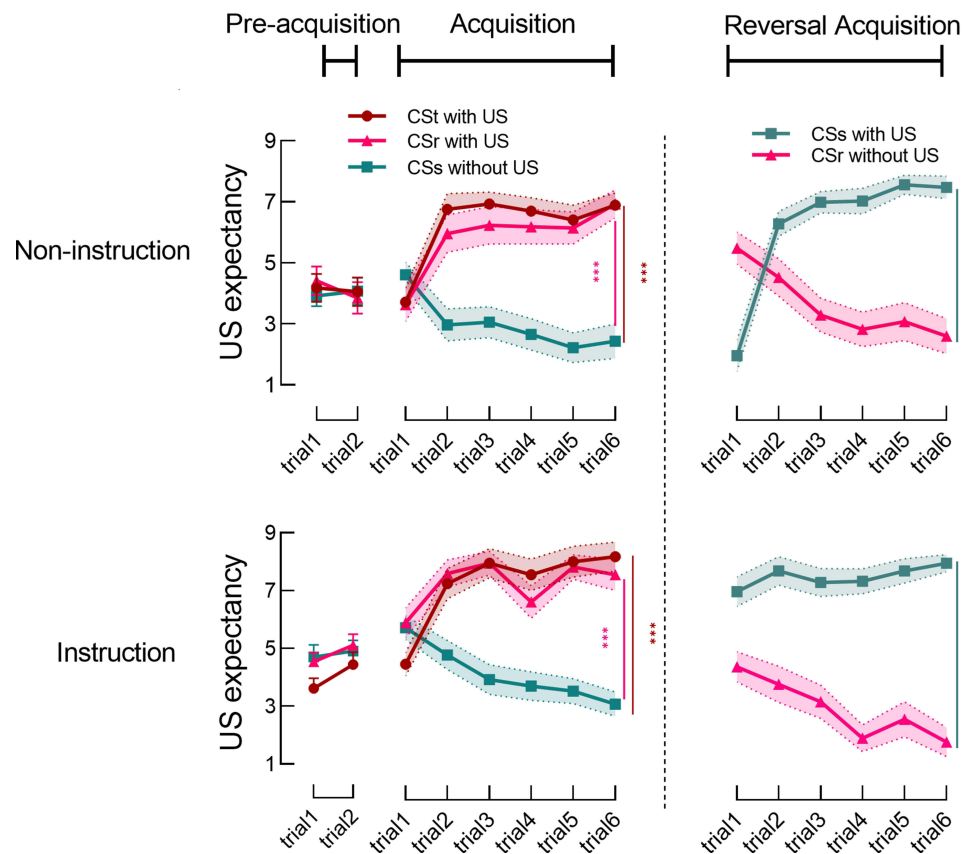


Figure 2 US expectancy ratings for CS.

Notes: Including three stages: pre-acquisition, acquisition, and reversal acquisition. During the acquisition stage, significant differences were observed in both groups for CS_t vs. CS_s and CS_r vs. CS_s, specifically at trial 6. Similarly, in the reversal acquisition stage, both groups displayed significant differences in US expectancy ratings between CS_s and CS_r. *** $p < 0.001$.

new CS-US associations in comparison with the instruction group. An interaction effect of trial $\times$ group was found in the mean difference values between NCS+ and NCS-, $F(5, 255) = 3.377$, $p = 0.006$, $\eta_p^2 = 0.062$, 95% CI [0.006, 0.110].

We also compared the mean US fear expectancy ratings for the final two trials between the acquisition and reversal acquisition stages. There was no three-way interaction of reversal $\times$ group $\times$ CS, $F(1, 51) = 0.357$, $p = 0.553$, $\eta_p^2 = 0.007$, indicating that both groups of participants exhibited consistent levels of associative learning for the stimuli during the later stage of acquisition, both before and after the reversal.

Fear Generalization

We calculated the mean US expectancy values for each stimulus across three trials during both the generalization and reversal generalization stages (see Figure 3). Subsequently, we performed a three-way mixed ANOVA, with reversal (before, after) $\times$ stimuli (GS1/NGS1–GS6/NGS6) $\times$ Group (non-instruction, instruction). No significant three-way interactions were found, $F(5, 255) = 0.239$, $p = 0.891$, $\eta_p^2 = 0.005$.

We extended our investigation by conducting mediation model analyses on the US expectancy ratings during the two generalization testing stages. Our findings showed that IU acted as a mediator of fear generalization responses from pre-reversal to post-reversal in the instruction group (see Figure 4A). The model fit indices in the instruction group were considered statistically significant, $p = 0.124$, $\chi^2/df = 1.121$, RMSEA = 0.051, CFI = 0.983, SRMR = 0.065. Conversely, the IU mediation effect failed to achieve an acceptable fit with the data in the non-instruction group (see Figure 4B), $p < 0.01$, $\chi^2/df = 1.963$, RMSEA = 0.159, CFI = 0.872, SRMR = 0.133, indicating a lack of adequate model fit. Furthermore, in the examination of the mediating effects of state anxiety and trait anxiety, we found that the indirect effects were not significant, $p_{\min} > 0.05$.

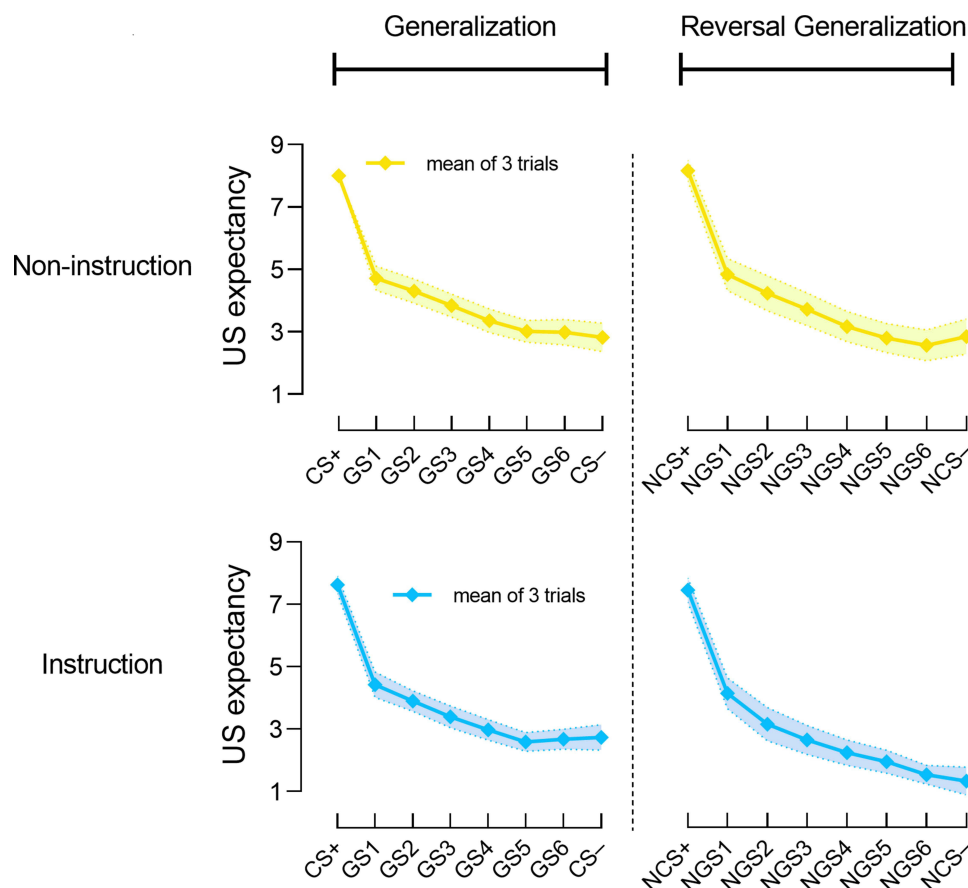


Figure 3 US expectancy ratings for the generalization and reversal generalization stages.

Notes: The mean US expectancy ratings across three trials for stimuli during both the generalization and reversal generalization stages. No significant differences in US expectancy ratings between GS and NGS stimuli were observed in either the non-instruction or instruction groups.

We analyzed the mediating effect of IU under the non-instruction and instruction conditions after computing mean fear expectancy ratings of six generalized stimuli. The model fit indices demonstrated a good fit, with $p = 0.848$, $x^2/df = 0.037$, CFI = 1.000, RMSEA < 0.001, and SRMR = 0.006. Further analysis showed significant differences in the mediating effect of IU across the groups, $\beta = 0.112$, $t(157) = 1.965$, $p = 0.049$, 95% CI [0.012, 0.234]. Thus, there were distinct patterns in the mediating effect of IU between individuals who received the instruction and those who did not. This substantiates the importance of considering the binary moderating variable of the presence or absence of instruction in explaining the differences in the mediating effect between the two groups. The detailed standardized coefficients of the mediating effects moderated by the instruction appear in [Supplementary Table 1](#).

We further investigated the discrepancies in the mediating effects of IU between the two groups within each GS–NGS (see [Supplementary Table 2](#)). Significant differences in the mediating effects between the two groups were observed during GS1–NGS1, indicating a stronger mediating effect of IU in the instruction group compared with the non-instruction group, $\beta = 0.142$, $t(157) = 2.247$, $p = 0.025$, 95% CI [0.036, 0.287]. The mediating effect of IU in the instruction group was present within the range of GS1–NGS1 and through GS4–NGS4, which is in close proximity to CS+ or NCS+. However, no significant IU mediating effect was detected for GS5–NGS5 and GS6–NGS6. For GS6–NG6, notable between-group variations became apparent in the direct effect of GS6 on NGS6, with the non-instruction group exhibiting a more pronounced direct effect than the instruction group, $\beta = 0.311$, $t(157) = 2.257$, $p = 0.024$, 95% CI [0.038, 0.571]. In the context of GS proximal to threat cue, participants in the instruction group demonstrated that the post-reversal generalization was mediated by IU. Specifically, individuals with elevated IU levels exhibited intensified fear generalization responses following the reversal. For GS proximal to safe cues, the non-instruction group tended to

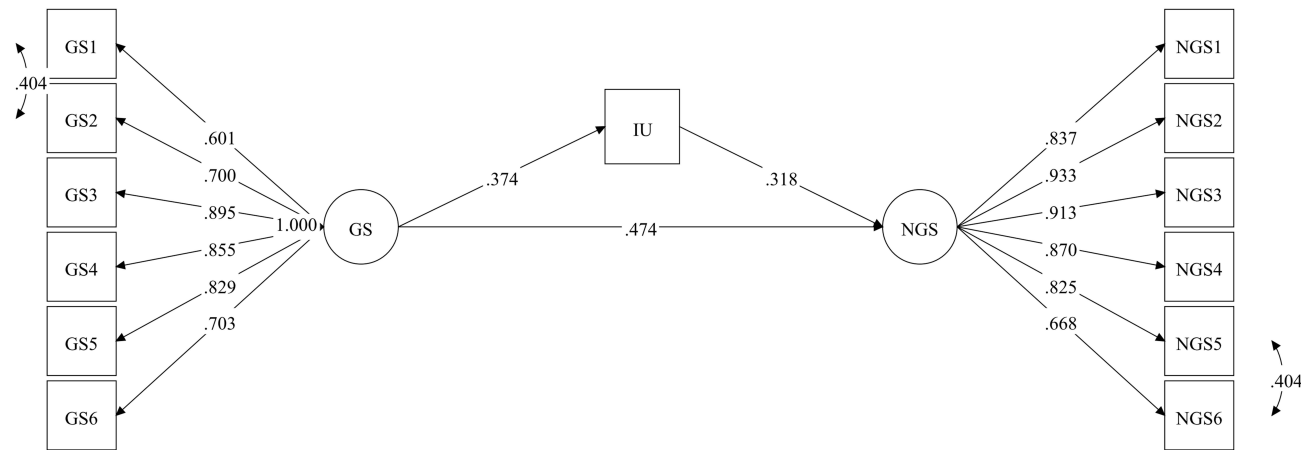
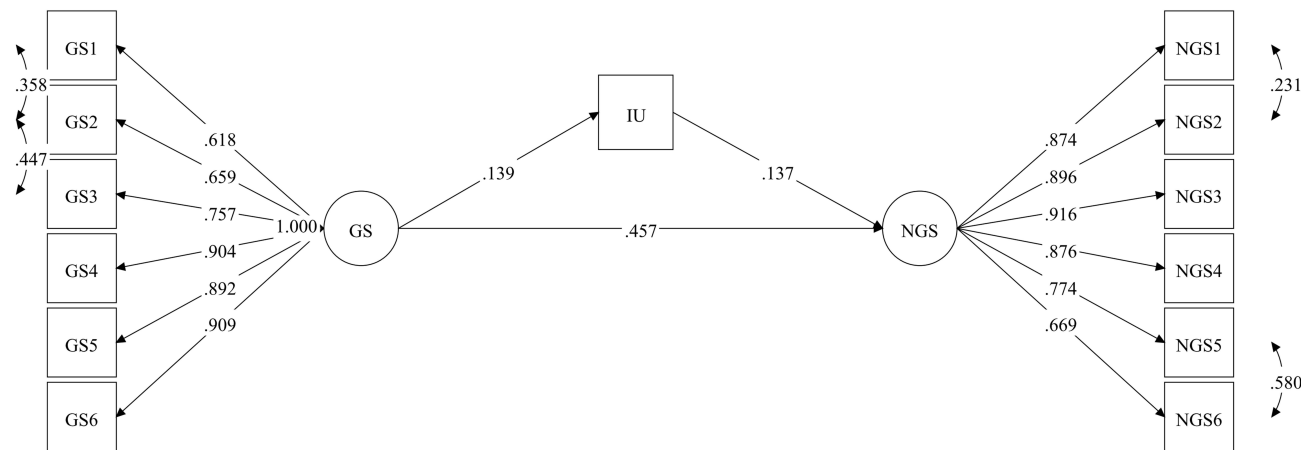
A**B**

Figure 4 The latent variable mediation pathways for both groups.

Notes: (A) represents the instruction group, with the path from GS to NGS through IU found to be significant. (B) represents the non-instruction group, with the same path not found to be significant.

carry over the generalized pattern from the pre-reversal to the post-reversal stage. In contrast, the instruction group demonstrated a pattern of updating associative learning, which facilitated the acquisition of fear expectancies from NCSs and the subsequent application of these acquired cues to evaluate fear expectancies for NGSs.

Regression analysis was performed on data from both groups to investigate the relationship between pre- and post-reversal fear generalization. IU was used as the mediating variable (see Figure 5). In the non-instruction group, no significant differences were observed between participants with high and low IU levels in the regression of pre-reversal fear generalization onto post-reversal fear generalization responses, $\beta = 0.246$, $t(77) = 1.000$, $p = 0.317$, 95% CI [-0.065, 0.977]. In the instruction group, participants with higher levels of IU exhibited a stronger fear expectancy of the US in the reversal generalization stage, compared with participants with lower levels of IU, $\beta = 1.624$, $t(80) = 2.529$, $p = 0.011$, 95% CI [0.530, 3.100]. Additionally, a significant difference between groups was observed, $\beta = 1.378$, $t(157) = 2.019$, $p = 0.043$, 95% CI [0.183, 2.864]. The results suggest that participants with low IU maintained the same level of US expectancy for NGS as participants with high IU in the non-instruction situation. In contrast, participants with low IU in the instruction group exhibited a reduced US expectancy for NGS during the post-reversal generalization test compared with participants with high IU.

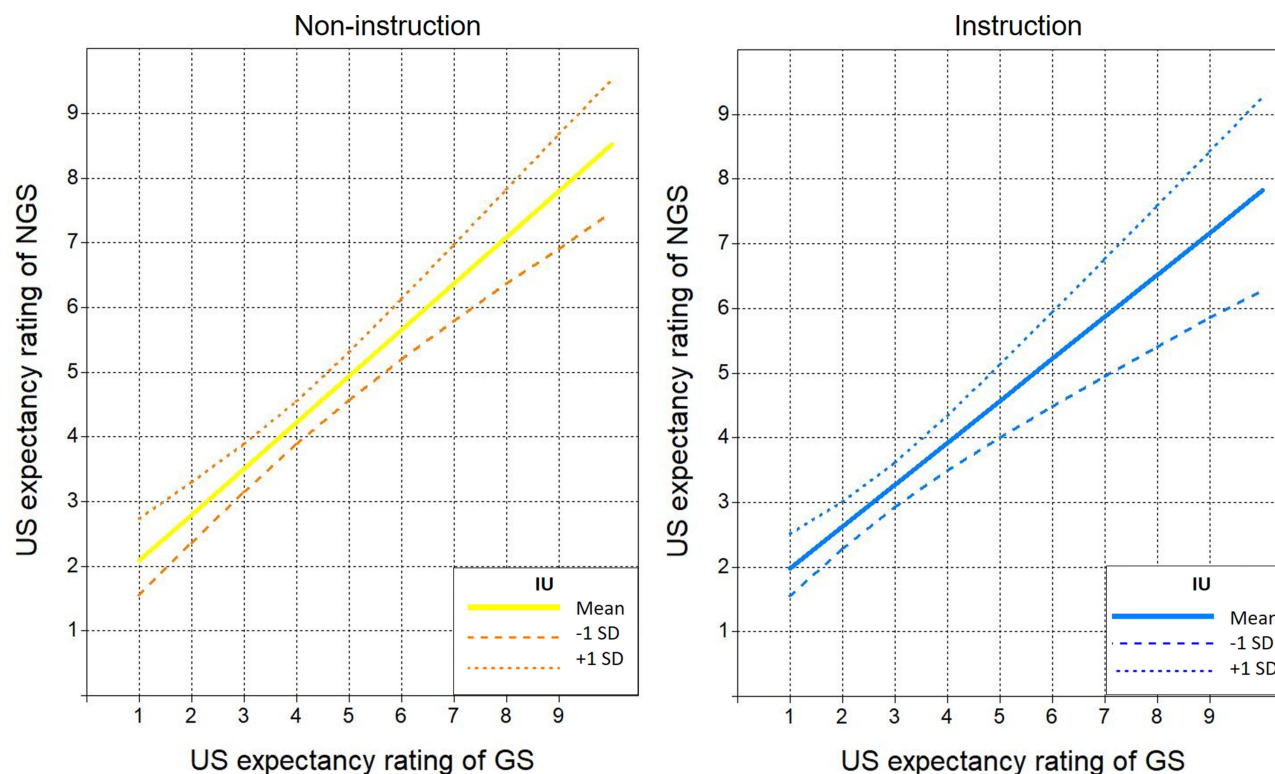


Figure 5 The regression lines of GS on NGS in two groups.

Notes: The regression lines for IU lower than 1 standard deviation (SD) below the mean and higher than 1 SD above the mean.

Discussion

This study investigated responses to fear generalization in individuals under instruction and non-instruction conditions. During the acquisition stage, participants in both groups demonstrated equivalent learning of the dual threat cues. However, during the early phase of reversal acquisition, participants in the non-instruction group showed delayed learning of the NCS-US association in the absence of reversal information, whereas participants in the instruction group showed accelerated learning in the presence of reversal information. This contrast was mainly evident in the temporal aspect of learning, with both groups displaying similar levels of NCS-US association learning during the later phase. Comparing fear generalization responses before and after reversal, a significant positive direct impact of pre-reversal fear generalization on post-reversal fear generalization was observed in both the non-instruction and instruction groups. In addition, we identified an indirect effect of IU in the instruction group, whereby IU levels indirectly influenced post-reversal fear generalization responses. In contrast, the non-instruction group did not exhibit such an indirect role of IU levels on post-reversal fear generalization responses. This highlights a significant disparity in the IU intermediary effect between the two groups.

Our study did not find a statistically significant distinction in US expectancy ratings for generalization stimuli between the two groups, which diverges from previous research.²⁷ In the non-instruction group, the post-reversal fear generalization test stage highlighted a greater tendency among individuals to interpret ambiguous stimuli as threatening under latent threat conditions.^{18,49} During this stage, individuals' generalization responses could be construed as adopting a "better safe than sorry" strategy.¹⁹ At this juncture, fear generalization responses did not differ significantly among individuals with varying levels of IU and anxiety. Even individuals with low IU and low anxiety levels displayed induced fear expectancies toward neutral stimuli in the absence of awareness regarding the CS-US reversal.

For the instruction group, disparities in post-reversal fear generalization responses among individuals with varying levels of IU can be comprehended through the lens of generalized unsafety (GU). GU theory challenges the notion that human learning progression involves a linear transition from anxiety-free states to anxious states solely through the acquisition of threat. Instead, it proposes a developmental trajectory that moves from heightened anxiety to reduced

specific anxiety by continuously learning about safe cues.⁵⁰ GU theory describes a propensity to maintain a stress-reactive state by default when explicit safety evidence is absent.^{49,51,52} Fear generalization responses in this framework are contingent on the absence of a perceived sense of safety rather than the mere perception of latent threats. The theory suggests that individuals under the influence of GU tend to maintain a state of vigilance in the absence of clear safety signals,⁵⁰ fostering fear responses that rely on the absence of perceived safety rather than the perception of potential threats. The observation of relatively subdued fear generalization responses among individuals with lower IU levels implies that individuals possess the ability to discern the safety of NGS during the post-reversal generalization test. This realization may stem from their previous experiences in generalization tests, where they came to understand that stimuli presented during the generalization test stage were entirely neutral, lacking any aversive properties and thus, resulting in GU inhibition. Individuals with higher IU levels displayed a distinctly different response. The absence of explicit NGS-US association information perpetuated their feelings of being unsafe. Despite awareness that no aversive shock would occur during the pre-reversal generalization test stage, recognizing the minimal threat value of the GS failed to effectively suppress GU responses.⁵⁰ This arose because of their inadequate sense of safety, which prevented efficient inhibition of GU reactions. These findings suggest that individuals with different IU levels may adopt distinct strategies when processing threat signals and perceiving safety.^{32,40}

Negative traits are widely considered as triggers for fear generalization. However, in this study, we only identified the mediating effect of IU and did not observe individual variations in fear responses to GS in conditions of uncertainty about the reversal for both state and trait anxiety. This contrasts with earlier research findings.^{10,33,35,36} A possible reason for the discrepancy may lie in the specific uncertainty conditions created by the experimental design used in our study. Previous research employing single stage fear generalization tests could have more readily elicited participants' latent expectations of potential threats, thus inducing anxiety rather than the particular sense of uncertainty that our experimental scenario entailed. Moreover, the robust connection between IU and anxiety suggests that heightened generalized responses could have been easily ascribed to anxiety. Our study outcomes imply that the relationship between IU and anxiety is intricate³³—while they do share common attributes in some settings, their specific manifestations could vary in certain contexts.

The notion that higher IU is linked to excessive fear generalization has been addressed in prior research, albeit with limited evidence substantiating the association between high IU and increased fear generalization.^{25,53} Our study, however, validates the proposition that heightened IU specifically correlates with a sensitivity to uncertainty, subsequently leading to fear generalization responses. Participants in the instruction group had ample opportunity to learn the association between GS and US during the pre-reversal fear generalization test stage, under the premise of theoretically intact safety learning and unimpaired ability for safety generalization.^{5,17} They knew that the GS posed no threat. However, our findings only demonstrated that individuals with lower IU levels were able to apply this learning experience effectively. These individuals exhibited a reduction in fear generalization toward the NGS after being informed of the new CS-US connection.

We propose that the contrast between fear generalization responses pre- and post-reversal is indicative of an active processing of uncertainty-related information by individuals with higher IU.⁵⁴ The experimental procedure appears to trigger a sensitivity to GU information in these individuals. After acquiring knowledge of the NCS-US association, they flexibly utilized this newfound associative learning to discriminate the threat value of NGS, demonstrating a proactive approach rather than passive processing. This behavior cannot be solely attributed to a high anxiety response to potential threats. Nevertheless, further research is required to understand the distinctiveness of the composition and expression of IU and anxiety. This will entail investigating the variations in their responses to coping with GU and potential threats, aiming to clarify their unique roles in the processes of conditional fear learning and emotional regulation.

This study offers new insights into the motivational and cognitive mechanisms that underlie sustained fear expectancy responses in individuals with high IU when confronted with generalized stimuli. Furthermore, it sheds light on the reasons for the suboptimal efficacy observed in clinical treatments or interventions targeting specific phobia patients, particularly those exhibiting a pronounced IU. Using exposure therapy as an illustrative example, despite patients acquiring a diminished fear response to specific phobic stimuli within the therapeutic setting, the persistence of heightened fear expectations when encountering diverse, uncertain situations outside the therapy room is probable. This discernment underscores the imperative for treatments to conscientiously consider individual differences^{1,35,39,41} and augment safety-learning generalization capacities.^{50–52} Importantly, the emphasis should extend beyond the

elimination of fear towards specific stimuli, encompassing the adaptability of treatment effects in real-world contexts. Such considerations can enhance the enduring and broadly applicable impact of therapeutic outcomes.

While this study produced important results, its inherent limitations should also be acknowledged. The study results indicate that, under conditions of heightened uncertainty within the non-instruction group, individuals with lower IU exhibited no discernible differential response in fear generalization after reversal compared with individuals with higher IU. We propose that individuals with lower IU may hold a heightened expectation of threats in this specific scenario, where the uncertainty associated with the threat may amplify or perpetuate their fear generalization responses. Although the study effectively guided participants by providing directed information to enhance their perception of threat associations—thereby increasing predictability and a sense of certainty – the absence of such guidance left participants in an uncertain and unpredictable state. However, our study design only allowed us to depict certainty and uncertainty as binary conditions. This limitation hampers our comprehensive understanding of the specific constituents of uncertainty and the precise impact of varying levels of uncertainty on fear generalization responses.³⁹ Hence, future research should integrate real-life situations to further quantify specific components of uncertainty. This approach would expand investigations into maladaptive fear generalization under conditions of uncertainty. Such endeavors will enhance our nuanced comprehension of the interplay between uncertainty and fear generalization.

Expanding the participant sample size is crucial. This study, while insightful, had a relatively modest sample size. Moreover, inclusion of participants with clinically-diagnosed anxiety-related disorders in future studies is essential. As they often exhibit excessive fear responses to non-threatening stimuli, and their sensitivity to uncertainty might differ from that of non-clinical populations.^{10,33} Comparison with the general population will lead to better understanding of the distinctions in uncertainty perception and fear generalization for patients, ultimately helping to refine interventions for clinical anxiety disorders. Such efforts may lead to a comprehensive understanding of the intricate relationships among uncertainty, mental health conditions, and fear generalization, offering robust evidence for clinical practice and guiding precise therapeutic interventions.

Conclusion

In summary, the present study employed a contingency reversal associative learning paradigm to investigate fear generalization under conditions of uncertainty. We examined the impact of IU on fear generalization in both non-instruction and instruction conditions. Our findings demonstrated that IU plays a mediating role in post-reversal fear generalization, specifically within the instruction group, surpassing the effects observed in the non-instruction group. Individuals with higher levels of IU exhibited heightened fear generalization toward the NGS even after being provided with specific CS-US associative information. This unique pattern of fear generalization expression underscores the intricate interplay between IU and fear generalization responses. These insights deepen our understanding of the precise role of IU in shaping fear responses under conditions of uncertainty, offering valuable perspectives for a broader spectrum of research concerning fear-related emotional phenomena.

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Disclosure

The authors report no conflicts of interest in this work.

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