An examination of intolerance of uncertainty and contingency instruction on multiple indices during threat acquisition and extinction training.

Abstract

Individuals who score high in self-reported Intolerance of Uncertainty (IU) tend to find uncertainty aversive. Prior research has demonstrated that under uncertainty individuals with high IU display difficulties in updating learned threat associations to safety associations. Importantly, recent research has shown that providing contingency instructions about threat and safety contingencies (i.e. reducing uncertainty) to individuals with high IU promotes the updating of learned threat associations to safety associations. Here we aimed to conceptually replicate IU and contingency instruction-based effects by conducting a secondary analysis of self-reported IU, ratings, skin conductance, and functional magnetic resonance imaging (fMRI) data recorded during uninstructed/instructed blocks of threat acquisition and threat extinction training (n = 48). Generally, no significant associations were observed between self-reported IU and differential responding to learned threat and safety cues for any measure during uninstructed/instructed blocks of threat acquisition and threat extinction training. There was some tentative evidence that higher IU was associated with greater ratings of unpleasantness and arousal to the safety cue after the experiment and greater skin conductance response to the safety cue during extinction generally. Potential explanations for these null effects and directions for future research are discussed.

Keywords: Acquisition, Extinction, Threat, Instructions, Intolerance of Uncertainty, Skin Conductance, fMRI

Introduction

The fear of the unknown is defined as ‘an individual’s propensity to experience fear caused by the perceived absence of information at any level of consciousness or point of processing’ (Carleton, 2016a, p. 5). The fear of the unknown is a lower order construct and is considered to underlie or be a sub facet of the higher-order construct of neuroticism, which relates to the experience of negative affect more broadly (Barlow, Sauer-Zavala, Carl, Bullis, & Ellard, 2014; Carleton, 2016a; Clark & Beck, 2011). By proxy, the fear of the unknown can be captured via self-reported Intolerance of Uncertainty (IU) (Carleton, 2016b), which measures the tendency to interpret and react to uncertainty negatively (Carleton, Norton, & Asmundson, 2007; Freeston, Rhéaume, Letarte, Dugas, & Ladouceur, 1994). Importantly, self-reported IU has been identified as a fundamental transdiagnostic dimension, as high levels of IU are found across a wide range of mental health disorders such as anxiety, trauma, and obsessive-compulsive disorders (Carleton et al., 2012; P. M. McEvoy, Hyett, Shihata, Price, & Strachan, 2019). Furthermore, several recent intervention and clinical studies have shed light on the potential of IU as a transdiagnostic treatment target (Dugas & Ladouceur, 2000; Oglesby, Allan, & Schmidt, 2017; Robichaud & Dugas, 2006; van der Heiden, Muris, & van der Molen, 2012) and trans-therapy change process (Peter M McEvoy & Erceg-Hurn, 2016). Due to this progress, there has been a surge in research examining the subjective, psychophysiological and neural basis of IU (for review see, Tanovic, Gee, & Joormann, 2018), in order to understand how IU-related mechanisms operate and can be targeted using therapeutic techniques (Shihata, McEvoy, Mullan, & Carleton, 2016).

Classical threat conditioning mechanisms have been typically examined to model the development and treatment of anxiety, trauma, and obsessive-compulsive

disorders (Craske, Treanor, Conway, Zbozinek, & Vervliet, 2014; Jacoby & Abramowitz, 2016; McNally, 2007; Pittig, Treanor, LeBeau, & Craske, 2018). An emerging body of research has identified IU as an important modulator of classical threat conditioning mechanisms (Lonsdorf & Merz, 2017; Morriss, Wake, Elizabeth, & van Reekum, 2021; Morriss, Zuj, & Mertens, 2021). While evidence is inconclusive on the role of IU in the initial learning of threat and safety associations during acquisition training (i.e. learning the pairing between a cue and an aversive outcome such as shock), there is ample evidence suggesting that IU disrupts updating of threat and safety associations during threat extinction training (i.e. learning that a pairing between a cue and aversive outcome such as shock no longer occurs) (for review see Morriss, Zuj, et al., 2021). More specifically, individuals with higher IU maintain the conditioned response during threat extinction training, indexed by greater skin conductance response and BOLD activity in the salience network (e.g. amygdala, insula, and ventromedial prefrontal cortex) to cues that no longer signal threat (Morriss, Christakou, & Van Reekum, 2015, 2016). Uncertainty related to the threat and safe contingencies during threat extinction training (i.e. the uninstructed removal of the aversive outcome such as a shock) is thought to maintain the conditioned response in individuals with high IU (Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, 2015; Morriss & van Reekum, 2019).

Contingency instruction can reduce uncertainty related to threat and safe contingencies, and subsequently speed up initial threat learning and extinction respectively (Luck & Lipp, 2016; Mertens, Boddez, Sevenster, Engelhard, & De Houwer, 2018). A few recent studies have demonstrated that individuals with high IU may be particularly sensitive to contingency instruction during classical threat conditioning procedures (Mertens & Morriss, 2021; Morriss, Bell, Biagi, Johnstone, &

van Reekum, 2021; Morriss & van Reekum, 2019). For instructed threat acquisition training with partial reinforcement, the results have been mixed for IU, with one study showing IU-related effects on ratings and neural activity (i.e. greater ventromedial prefrontal cortex activity to the learned threat versus safety cue; Morriss, Bell, et al., 2021) and another study not showing any IU-related effects on ratings, auditory startle blink and skin conductance response (Mertens & Morriss, 2021). Notably, across several experiments, explicit instructions about the future absence of CS-US pairings have been found to promote extinction of the conditioned response in individuals with high IU indexed by a reduction in differential skin conductance response to the learned threat versus safety cues (Morriss & van Reekum, 2019). While these results are promising and highlight the potential malleability of IU-related biases through contingency instruction, further conceptual replication is warranted to address whether IU- and contingency instruction-related effects are reliable across neural and psychophysiological measures.

To examine IU- and contingency instruction-based effects, we conducted a secondary analysis of a threat conditioning experiment with uninstructed and instructed blocks of threat acquisition and extinction training. Multiple read-out measures (i.e. arousal and valence ratings, skin conductance, and functional magnetic resonance imaging) and the self-reported Intolerance of Uncertainty Scale (IUS: Freeston et al., 1994) were assessed. We used visual shape stimuli as conditioned stimuli and mild electric shocks as unconditioned stimuli, in line with prior research (Lonsdorf et al., 2017).

We hypothesised:

(1) IU may be significantly associated with valence and arousal ratings of CSs assessed before and after the threat learning experiment. However, given the mixed results from the prior literature on IU and ratings (for review see Morriss et al., 2021), a directional hypothesis is not specified.

(2) IU may be significantly associated with skin conductance response during *uninstructed* and *instructed* threat acquisition training. Because prior research has shown both a negative (Sjouwerman et al., 2020) and a positive relationship (Starita et al., 2019) between IU and skin conductance responding to learned fear vs. safety cues, a directional hypothesis is not specified.

(3) IU may be significantly associated with neural activity during *uninstructed* and *instructed* threat acquisition training. However, given the limited literature on IU and neural activity during acquisition training, a directional hypothesis is not specified.

(4) Higher IU will be significantly associated with greater skin conductance responding and BOLD activity in the salience network (i.e. amygdala, insula, ventromedial prefrontal cortex) to learned threat vs. safe cues during *uninstructed* threat extinction training, similar to previous research (Morriss, Wake, et al., 2021; Morriss, Zuj, et al., 2021).

(5) IU may be significantly associated with skin conductance responding or BOLD activity in the amygdala and insula during *instructed* threat extinction training (Morriss & van Reekum, 2019). Again, given the limited literature on IU and neural activity during instructed threat extinction training, a directional hypothesis is not specified.

(6) IU may be significantly associated with greater ventromedial prefrontal cortex activity during *instructed* threat extinction training (Morriss et al., 2015; Morriss, Bell, et al., 2021).

Please note that the hypotheses above deviate from the original preregistered hypotheses on OSF (osf.io/6asz2) due to an error by the second author, whereby the second author did not update the wording of the hypotheses after extensive debate with another lab group. More specifically, some of the hypotheses above were rephrased as non-directional (e.g. 'may be significantly associated) rather than directional (e.g. 'no significant association'). The amended hypotheses are in line with another manuscript on a similar topic for the special issue (https://psyarxiv.com/hj5gt/).

Method

The hypotheses were tested using secondary analyses of a data set collected to examine the association between heart rate variability (HRV) and the extinction of conditioned fear (in preparation). These secondary analyses of this data set were registered at OSF registries (osf.io/6asz2).

Participants

Forty-eight participants (27 women, 21 men; mean age = 22.3 years (SD = 2.7); Ethnicity: 48 White) completed the threat learning task in the MRI scanner. Participants were selected from a sample of 144 University students (mean age (SD)

= 22.9 (2.87)) who were screened for their resting HRV levels and fulfilled the following criteria: They were right-handed, aged between 18 and 30 years, had a body-mass-index (BMI) between 18 and 30, smoked less than ten cigarettes a day, performed no competitive sports in the last two years, had intact color vision and no loss of hearing, no self-reported neurological, cardiovascular and mental disorders, no current medication affecting the central or the parasympathetic nervous system, were not pregnant and not claustrophobic and had no ferromagnetic implants or tattoos in the head and neck area. Dropouts from the analyses of different depending variables are described in the *Measurements* section.

Because this is a secondary analysis, a sensitivity power analysis was conducted to assess whether the sample size would provide sufficient power to detect predicted effects. The sensitivity power analysis was based on a correlation: point biserial model, in line with similar statistical tests (e.g. correlations between IU and CS+ - CS- difference scores) reported in a meta-analysis of IU-related effects on skin conductance response during threat extinction training (Morriss, Wake, et al., 2021). Based on our largest (one tailed, $\alpha = 0.05$, $1 - \beta$ err prob = 0.8, $n = 48$) and smallest (two tailed, $\alpha = 0.05$, $1 - \beta$ err prob = 0.8, $n = 35$) sample size, the effect sizes that we were able to detect were between 0.34-0.43. These effect sizes are a little larger than the effect sizes (0.25-0.31) reported for a recent meta-analysis of IU-related effects on skin conductance response during threat extinction training (Morriss, Wake, et al., 2021). Despite the study being underpowered to detect individual differences in IU, the study still provides an opportunity to examine the relationships between IU and different indices of threat acquisition and extinction training, in particular that of neural indices which are currently rare in the IU literature.

Materials

Geometric figures, a blue square and an orange circle, served as conditioned stimuli (CS) and were presented for 6000 ms. Assignment of geometric figures to CS+ or CS- was counterbalanced between participants. The electrotactile stimulus, which served as an unconditioned stimulus (US), was an electric pulse train with a duration of 500 ms (100 single pulses) and began 5500 ms after CS+ onset, i.e., both co-terminated together. The US was generated with a DSA7 Digitimer (Fa. Digitimer Ltd, Hertfordshire, UK) and applied to the left lower leg using MRI-compatible reusable Ag cup electrodes (10 mm; Fa. Medical Products, Wiesbaden, Germany). For each participant, the US was adjusted to a level that they described as “unpleasant, but not painful” in a step-by-step procedure that began with a low-intensity, barely perceptible stimulus. Its mean physical intensity was 9.4 (SD = 4.0) mA.

Procedure

We determined the participant’s eligibility for study participation via a telephone interview. If eligible, they were asked to fill out an online survey with several questionnaires, including the German version of the Intolerance of Uncertainty Scale and the German version of the State-Trait-Anxiety-Inventory, and invited to a screening session to assess their heart rate variability (HRV) levels. Participants with high or low HRV levels were then invited to take part in a threat learning task in the MRI unit of the Greifswald University Hospital approximately one week later.

After arrival at the MRI unit, participants were informed about the upcoming procedures and completed a consent form. Afterward, ECG electrodes were attached, and they performed a Vanilla task during which their resting HRV was

assessed. Then, we attached the EDA electrodes and placed the participants in the MRI scanner. Inside the scanner, participants rated the perceived valence and arousal of the geometric figures serving as CS. After running a localizer and a resting state sequence, we individually calibrated the aversive US to achieve a level that was “unpleasant, but not painful”. Then, participants performed the threat learning task and underwent a T1-weighted sequence directly afterward. We asked participants about their awareness about CS-US contingencies and to again rate valence and arousal of the CS and, finally, they underwent a DTI sequence.

Threat learning task

Before the threat learning task, the participants were informed that they would now see the geometric figures they had just judged in terms of valence and arousal, that the electrotactile stimulus would also appear from time to time, and that it might be possible to establish a connection between the occurrence of the electrotactile stimulus and the geometric figures. During the subsequent acquisition training, 50% of the 16 CS+ trials co-terminated with the US while the 16 CS- presentations were never paired with the US (see Figure 1). Inter-trial intervals (ITIs) varied between 4000-8000 ms. Four different orders ensured that acquisition training began with CS+ and CS- trials equally often between participants. After half of the acquisition trials, a slide informed participants that “if an aversive stimulus is applied, then only with this symbol: blue square/ orange circle” (instructed acquisition). During extinction training, 16 CS+ and 16 CS- were presented without any reinforcement. After half of the extinction trials, a slide informed participants that “no further aversive stimuli will be applied in subsequent trials” (instructed extinction).

[Insert Figure 1 about here]

Measurements

Questionnaire: We administered the 27-item German version of the Intolerance of Uncertainty Scale (IUS; Gerlach, Andor & Patzelt, 2008) to assess individual differences in aversion to uncertainty.

Ratings: Participants rated the valence and arousal of the CS before and after the threat learning task using the Self-Assessment Manikin (SAM; Bradley & Lang, 1994) with 9-point Likert scales. The scales were anchored with adjectives pleasant and unpleasant for the valence rating and with calm and arousing for the arousal rating. From two participants, rating data from before the threat learning task are missing due to investigator error.

SCR magnitude: Electrodermal activity (EDA) was assessed using two MRI-compatible reusable Ag/AgCl electrodes (4 mm; Fa. Brain Products, Gilching, Germany) filled with a 0.05M sodium chloride electrolyte medium. The electrodes were placed adjacently on the hypothenar of the participant's non-dominant hand and connected to a GSR-MR module and the BrainAmp ExG system. The signal was evoked by applying a constant voltage of 0.5 V, amplified, filtered with a low cutoff time constant of 10s and high cutoff frequency of 250 Hz, and recorded with a sampling rate of 5000 Hz via the BrainVision Recorder software (all Brain Products,

Gilching, Germany). EDA data are missing from eight participants due to equipment failure or non-removable MRI artefacts.

EDA data were down-sampled to 10 Hz using BrainVision Analyzer (Brain Products, Gilching, Germany) and further processed using a custom-made script. Skin conductance response (SCR) amplitudes were scored from trough to peak as the first increase starting between 0.9 and 4.0 s after CS onset and a minimum response amplitude of 0.01 µS. Trials in which these response criteria were not fulfilled were classified as non-responses and scored as zeros (62.9%), trials with excessive baseline activity or artefacts as missings (0.7%). Five participants were excluded from further analyses, because they did not show SCRs to the US in more than 50% of trials (Lonsdorf et al., 2019).

The resulting values were subjected to a square root transformation in order to cope with the regularly skewed distribution of SCRs (Boucsein et al., 2012) and standardized as a proportion of the maximal response to correct for inter-individual variance in amplitudes (Braithwaite et al., 2013). For the CS+ and the CS- trials, the values were then averaged over each of the eight trials during uninstructed acquisition, instructed acquisition, uninstructed extinction, and instructed extinction.

fMRI: Functional and anatomical brain images were recorded using a 3T Siemens Magnetom Verio scanner with a 12-channel head coil. Before functional imaging, a gradient echo sequence (TR = 488 ms, TE1 = 4.92 ms, TE2 = 7.38 ms, flip angle = 60°) was conducted to construct a fieldmap for the unwarping procedure and a shimming sequence to optimize field homogeneity. During the threat learning task, 404 echo-planar images were acquired in a transversal direction at a 20° angle to the

AC-PC-line (33 slices, voxel size = 2 x 2 x 3 mm, 1 mm gap, TR = 2000 ms, TE = 23 ms, matrix = 104 x 104 mm, flip angle = 70°). Afterward, a T1-weighted anatomical volume (GRAPPA, 176 sagittal slices, voxel size 1 x 1 x 1 mm, TR = 1690 ms, TE = 2.52 ms, matrix = 256 x 256 mm, flip angle = 9°) was recorded.

We preprocessed and analysed the MRI data with SPM12 (Functional Imaging Laboratory, Wellcome Trust Centre for Neuroimaging, London, UK). Raw anatomical and functional images were inspected visually for morphological anomalies, recording errors, artefacts, and pronounced movements. Six participants were excluded from further analyses (one with enlarged ventricles, one due to recoding error, four with movements > 0.5 mm per TR in more than 5% of volumes). Further preprocessing steps of the functional images included realignment to account for participant movements and unwarping by using a fieldmap to correct image deformations due to magnetic field inhomogeneities. Then the functional images were co-registered with the anatomical T1 volume, spatially normalized via segmentation, and smoothed with 6 mm FWHM.

The preprocessed functional images were entered into a 1st level model with eight regressors of interest (CS+$_{unpaired}$, and CS-$_{unpairedmatch}$ during uninstructed acquisition and instructed acquisition and CS+ and CS- during uninstructed extinction and instructed extinction), and six additional regressors of no interest (CS+$_{paired}$ and CS-$_{pairedmatch}$ during uninstructed acquisition and instructed acquisition, the instruction slides, US presentations, and six movement regressors). CS+$_{unpaired}$ regressors contained the four CS+ presentations during each of the acquisition phases during which the CS+ did not co-terminate with the US. The regressor CS-$_{unpairedmatch}$ contained the same number of CS- presentations at comparable time periods.

CS regressors contained a stimulus onset function (6000 ms) convolved with a canonical hemodynamic response function (HRF) as implemented in SPM12, the US regressor a stick function convolved with the same HRF.

Analyses

Ratings: To examine IU-related differences on ratings of the CS before and after the threat learning task we used two separate 2 x 2 repeated measures ANCOVAs as implemented in IBM SPSS Statistics (Version 27). Within-factors were CS type (CS+, CS-) and Time (PRE-, POST-learning task) and the total IUS score was included as a mean centered covariate. Partial ETA squared (η_p^2) is reported as a measure of effect size.

SCR magnitude: To examine IU-related differences on SCR during uninstructed and instructed acquisition, we used a 2 x 2 repeated measures ANCOVA as implemented in IBM SPSS Statistics (Version 27). Within-factors were CS type (CS+, CS-) and Instruction (No Instruction, Instruction) and the total IUS score was included as a mean centered covariate. Partial ETA squared (η_p^2) is reported as a measure of effect size.

To examine IU-related differences on SCR during uninstructed and instructed extinction, we used Pearson correlation analysis with IUS scores and SCR CS+/CS- difference scores during uninstructed and instructed extinction training.

fMRI: To examine IU-related differences on BOLD activity, we used 2nd level one-sample t-tests as implemented in SPM12 on the 1st level contrasts CS+ > CS- and the total IUS score was included as a mean centered covariate for each of the experimental phases (uninstructed acquisition, instructed acquisition, uninstructed extinction, instructed extinction). Region-of-Interest (ROI)-analyses were conducted with a significance threshold of $p < 0.05$ corrected for multiple comparisons (family wise error rate; FWE) within masks derived from the Wake Forest University PickAtlas (Maldjian et al., 2003) for the left (146 voxels) and right (169 voxels) amygdala, the left (855 voxels) and right (769 voxels) anterior insula, and the left and right (1061 voxels) ventromedial PFC.

Results

In the main manuscript, only associations of IUS scores with different indicators of threat acquisition and threat extinction are reported. Main effects and interactions of the experimental parameters can be found in the supplement.

Questionnaire

IUS scores were normally distributed and typical for a community sample [IUS: *M* = 56.0, *SD* = 16.0; see Figure 2].

[Insert Figure 2 about here]

Ratings

As expected, we found no significant associations between IUS and valence and arousal ratings before and after the threat learning experiment (Valence: Main Effect IUS: F(1,44) = 1.13, p = .293, η_p^2 = .025; CS type x IUS: F(1,44) = 0.43, p = .514, η_p^2 = .010; Time x IUS: F(1,44) = 1.65, p = .206, η_p^2 = .036; CS type x Time x IUS: F(1,44) = 0.20, p = .661, η_p^2 = .004); Arousal: Main Effect IUS: F(1,44) = 1.82, p = .184, η_p^2 = .040; CS type x IUS: F(1,44) = 1.10, p = .301, η_p^2 = .024; Time x IUS: F(1,44) = 0.23, p = .637, η_p^2 = .005; CS type x Time x IUS: F(1,44) = 0.02, p = .891, η_p^2 = .000)).

[Insert Figure 3 about here]

However, Figure 3 indicates less CS+/CS- differentiation of valence and arousal ratings in individuals with high IUS levels after the threat learning task due to higher unpleasantness and arousal ratings of the CS-. Thus, we performed exploratory Pearson correlation analyses and found that higher IUS scores were associated with ratings of the CS- as more unpleasant (r(48)= .286, p < .05, 95% CI [.002, .527]) and, on a trend level, also more arousing (r(48) = .270, p = .06, 95% CI [-.015, .515]) after the threat learning task. After correction for multiple comparisons, however, these associations would no longer be considered significant in our sample.

Threat acquisition training

SCR magnitude: Unexpectedly, we found a significant association between IUS and SCR magnitudes during uninstructed versus instructed acquisition training (Instruction x IUS: F(1,33) = 6.40, p < .05, η_p^2 = .16). Follow-up Pearson correlation analysis confirmed that higher IUS scores were associated with lower differences

between SCRs during uninstructed and instructed acquisition training (r(35) = -.403, p < .05, 95% CI [-.649, .-.081]); Figure 4A. We did not find any other significant associations between IUS and SCR magnitudes during acquisition training (Main Effect IUS (tested with unstandardized SCR values): F(1,33) = 0.18, p = .671, η_p^2 = .006; CS type x IUS: F(1,33) = 0.17, p = .682, η_p^2 = .005; CS type x Instruction x IUS: F(1,33) = 2.19, p = .148, η_p^2 = .062).

[Insert Figure 4 about here]

fMRI: IUS scores were not significantly associated with differential BOLD activity in the salience network (amygdala, anterior insula, and ventromedial PFC) during uninstructed and instructed threat acquisition training (Table 1).

Table 1: ROI-analysis of associations between Intolerance of Uncertainty scores and $CS+_{unpaired}$ vs $CS-_{unpairedmatch}$ responses during threat acquisition training.

Region	Side	MNI-coordinates			t	p_{FWE}	MNI-coordinates			t	p_{FWE}
		x	y	z			x	y	z		
		Uninstructed threat acquisition training									
		Pos. corr. with CS+ > CS-					**Neg. corr. with CS+ > CS-**				
Amygdala	L	-28	0	-27	0.66	.871	-24	-2	-18	2.31	.323
	R	30	4	-27	1.96	.520	24	2	-15	2.19	.408
Anterior Insula	L	-30	16	6	2.04	.907	-38	4	6	3.64	.117
	R	42	22	-9	1.64	.970	38	8	6	3.31	.215
Ventromedial PFC	L	0	16	-24	3.23	.299	0	56	-21	2.58	.699
	R	2	18	-21	2.89	.509	14	44	-12	2.44	.788
		Instructed threat acquisition training									
		Pos. corr. with CS- > CS+					**Neg. corr. with CS- > CS+**				
Amygdala	L	-24	4	-18	1.46	.705	-24	-4	-12	1.87	.534
	R	34	4	-21	1.14	.824	32	-4	-21	1.94	.539
Anterior Insula	L	-40	22	0	3.17	.294	-32	0	12	3.81	.081
	R	44	18	-12	2.56	.648	50	6	-3	3.04	.350
Ventromedial PFC	L	-10	48	-6	3.55	.166	-8	14	-21	1.53	.988
	R	2	40	-6	2.84	.553	16	66	-9	2.91	.502

Threat extinction training

SCR magnitude: Pearson correlation coefficients between IUS scores and CS+/CS- SCR difference scores were negative, not significant, and comparable in effect size for uninstructed (r(35) = -.262, p = .128, 95% CI [-.548, .078]) and instructed (r(35) = -.235, p = .174, 95% CI [-.527, .107]) extinction training. As evident from Figure 4B and confirmed by exploratory Pearson correlation analyses, the observed negative coefficients stem from a positive association between higher IUS scores and higher SCR magnitudes to the CS- (r(35) = .341, p = .045, 95% CI [.009, .605]). After correction for multiple comparisons, however, this association would no longer be considered significant in our sample

fMRI: IUS scores were not significantly associated with differential BOLD activity in the salience network (amygdala, anterior insula, and ventromedial PFC) during uninstructed threat extinction training and also not with differential BOLD activity in the ventromedial PFC during instructed threat extinction training (Table 2).

Table 2: ROI-analysis of associations between Intolerance of Uncertainty scores and $CS+_{unpaired}$ vs $CS-_{unpairedmatch}$ responses during threat EXTINCTION training (t values > 3 reported)

Region	**Side**	**MNI-coordinates**			**t**	**p_{FWE}**	**MNI-coordinates**			**t**	**p_{FWE}**
		x	**y**	**z**			**x**	**y**	**z**		
		Pos. corr. with CS+ > CS-					**Neg. corr. with CS+ > CS-**				
Amygdala	L	-28	-6	-18	2.05	.441	-24	-8	-12	1.06	.811
	R	32	-4	-21	2.43	.299	20	6	-18	1.19	.811
Anterior Insula	L	-32	10	-15	3.25	.252	-26	24	-6	1.88	.944
	R	30	12	-15	3.41	.176	48	8	6	1.23	.991
Ventromedial PFC	L	-4	20	-21	3.62	.141	-10	14	-21	1.95	.945
	R	4	60	-3	3.15	.348	16	48	-6	2.33	.840
		Instructed threat extinction training									
		Pos. corr. with CS- > CS+					**Neg. corr. with CS- > CS+**				
Amygdala	L	-24	-2	-27	1.44	.689	-16	0	-15	2.49	.237
	R	26	2	-27	1.77	.589	32	-8	-12	2.82	.109
Anterior Insula	L	-40	22	0	1.82	.944	-44	2	-6	2.30	.787
	R	36	12	-12	2.94	.874	26	16	-18	1.96	.905
Ventromedial PFC	L	-6	24	-21	2.62	.648	0	56	-21	2.80	.529
	R	10	20	-15	2.77	.561	6	22	-27	3.25	.277

Discussion

In the current study, we investigated the impact of self-reported IU on ratings, skin conductance, and functional magnetic resonance imaging (fMRI) data recorded

during uninstructed/instructed blocks of threat acquisition and threat extinction training. Generally, no significant associations were observed between self-reported IU and differential responding to learned threat and safety cues for any measure during uninstructed/instructed blocks of threat acquisition and threat extinction training. There was some weak evidence that higher IU was associated with greater ratings of unpleasantness and arousal to the safety cue after the experiment and greater skin conductance response to the safety cue during threat extinction overall. Such results provide further discussion on the role of IU and contingency instruction on subjective, physiological and neural measures during threat acquisition and extinction training.

The experiment yielded typical effects of conditioning across subjective, physiological, and neural measures for the uninstructed/instructed threat acquisition and extinction training phases (Fullana et al., 2016, 2018; Luck & Lipp, 2016; Mechias et al., 2010; Mertens et al., 2018) (see Supplementary Material). Interestingly, no significant associations were observed between IU and differential responding to learned threat and safety cues for any measure during uninstructed and instructed threat acquisition and extinction training. The lack of IU-related effects for uninstructed/instructed threat acquisition training follows prior research (for review, see Morriss, Zuj et al., 2021; c.f. Morriss et al., 2021). However, the lack of IU-related effects for uninstructed/instructed threat extinction training is somewhat surprising, given: (1) the multitude of studies that have reported how high levels of IU maintain the conditioned response (i.e. differential skin conductance response and amygdala activity to learned threat versus safety cues) during uninstructed threat extinction training (for meta-analysis and review see, Morriss, Wake et al., 2021; Morriss, Zuj et al., 2021) and (2) the recent work that has shown how the conditioned

response (i.e. differential skin conductance response to learned threat versus safety cues) can be reduced in individuals with higher IU through instructed threat extinction training (Morriss & van Reekum, 2019). The study did reveal some tentative evidence that higher IU was associated with greater ratings of unpleasantness and arousal to the safety cue after the experiment and greater skin conductance response to the safety cue during threat extinction training overall. These findings are in line with a few previous studies that have found that individuals with high IU display larger skin conductance responses to safety cues during uninstructed threat extinction training (Morriss et al., 2015, 2016) and larger auditory startle blink to safety cues generally (Morriss, Bennett, & Larson, 2021). Although further research is warranted to ascertain the stability of IU-related effects upon safety cues during threat extinction training (see recent meta-analysis by Morriss, Wake et al., 2021).

The IU-related null effects observed in this study may be best explained by different experimental design choices. Firstly, general contingency instructions were provided before the task began (i.e. 'it might be possible to establish a connection between the occurrence of the electrotactile stimulus and the geometric figures') and precise contingency instructions were presented throughout the task to all participants (within-subject design). Prior research that has found interactions between IU and contingency instruction have used more extreme contingency instruction shifts to maximise perceptions of uncertainty (i.e. no instruction to precise instruction or vice versa) and used a control group with no instructions (between-subject design) (Mertens & Morriss, 2021; Morriss & van Reekum, 2019). As an additional consequence of the within-design, instruction effects are confounded with other effects such as the individual learning history and general habituation (cf. Lonsdorf et al., 2017) which may affect the power to detect effects of individual

differences. Secondly, in the current study, there were more instruction periods (i.e. 3), compared to previous research (I.e. 2; Morriss & van Reekum, 2019), which may result in more familiarity with the experimental task and reduce uncertainty related to the contingencies overall. Thirdly, the number of trials presented between instruction periods was fewer (i.e. 8 trials of each CS), compared to prior work demonstrating that IU-related distress during threat associative learning, particularly during extinction learning, is best captured with more trials (e.g. 12-16 trials of each CS) (see meta-analysis by Morriss, Wake et al., 2021). Fourthly, for this study, the sample was selected based on strict criteria (e.g. BMI, free of neurological, cardiovascular, and mental disorders, etc), while the majority of past research on IU and threat conditioning has been less restrictive (e.g. based on age range and/or psychotropic medication usage) (Flores et al., 2020; Lucas et al., 2018; Morriss et al., 2015; Wake et al., 2021; Zuj et al., 2020; but c.f. San Martin et al., 2020; Sjouwerman et al., 2020). Relatedly, the mean IUS score in the current study is lower (M = 56) than the majority of past studies in the literature (M > 62) (see supplement in the meta-analysis by Morriss, Wake et al., 2021), resulting in less variability in mean IUS, particularly at the higher end of the scale. Lastly, the study was underpowered compared to prior research on IU and skin conductance response during threat extinction training (Morriss, Wake, et al., 2021). Thus, caution is warranted regarding the interpretation of the observed null results. Despite these differences, however, it is also possible that the null effects occurred by chance (Schimmack, 2012).

In sum, we failed to conceptually replicate IU-related effects on psychophysiological and neural measures during uninstructed and instructed threat extinction training. The lack of IU-related effects on psychophysiological and neural measures in this study was likely due to differences in experimental design and

sampling. Further research is required to understand the influence of IU and contingency instruction on threat acquisition and extinction training, preferably in larger samples and using tasks with more extreme contingency shifts, more trials, and between-subject designs.

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