Your guess is as good as mine: A registered report assessing physiological markers of fear and anxiety to the unknown in individuals with varying levels of intolerance of uncertainty

Jayne Morriss*, Nico Biagi, & Helen Dodd,

Centre for Integrative Neuroscience and Neurodynamics

School of Psychology and Clinical Language Sciences

University of Reading

Reading

UK

* Correspondence:

Jayne Morriss

Centre for Integrative Neuroscience and Neurodynamics

j.e.morriss@reading.ac.uk

Abstract

Individuals who score high in Intolerance of Uncertainty (IU) have a tendency to find uncertainty and the unknown aversive. However, there is a dearth of literature on the extent to which the known vs. the unknown during threatening contexts induce fear and anxiety in individuals with high IU. In the following registered report we attempted to address this question by manipulating the known and unknown in the threat of predictable and unpredictable aversive events task. Throughout the task, we measured a variety of self-report (ratings of valence and arousal) and physiological indices (skin conductance, pupil dilation, orbicularis oculi, corrugator supercillii). We collected data from 93 participants. Higher IU, relative to lower IU was associated with: (1) less discriminatory orbicularis oculi activity between cue and interstimulus interval periods across conditions, and (2) larger corrugator supercillii activity to the known predictable shock condition and smaller corrugator supercillii activity to the known unpredictable shock condition, compared to the other conditions. These findings provide evidence that IU-related biases manifest differently depending on the physiological marker of fear and anxiety and the type of known-unknown threat i.e. orbicularis oculi activity was related to generalisation across conditions, whilst corrugator supercillii activity reflected distress/relief during conditions with known threat. Ultimately, this research will inform future models of IU in relation to anxiety and stress disorders.

Keywords: Threat, Uncertainty, Unknown, Intolerance of Uncertainty, Skin Conductance, Pupil Dilation, Electromyography

Introduction

Fear of the unknown is '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). It is considered a basic component of anxiety and negative affect more broadly (Carleton, 2016b). Fear of the unknown is captured in self-reported Intolerance of Uncertainty (IU) (Carleton, 2016a, 2016b; Freeston, Rhéaume, Letarte, Dugas, & Ladouceur, 1994). Based on a recent definition: 'IU is a dispositional incapacity to endure the aversive response triggered by the perceived absence of salient, key, or sufficient information, and sustained by the associated perception of uncertainty' (Carleton, 2016b; p. 31). Individuals with high self-reported IU are prone to a range of mental health disorders (Boulter, Freeston, South, & Rodgers, 2014; Carleton et al., 2012; Gentes & Ruscio, 2011; McEvoy & Mahoney, 2012). Over the last decade, the study of IU has gained momentum and now sits at the forefront of anxiety research (Carleton, 2016a, 2016b; Shihata, McEvoy, Mullan, & Carleton, 2016).

Carleton's (2016b) definition suggests that perceiving the unknown is the ultimate stressor or threat for individuals with high IU. In order to perceive that something is unknown, then one must know that they don't know. However, it still remains an open question as to what quantity of information has to be perceived as absent or unknown to elicit fearful or anxious responding in individuals with high IU. Do individuals high in IU only experience fear and anxiety during threatening contexts when there is something unknown in relation to something known (i.e. knowing some information but perceiving there to be some other information missing) or do they also experience fear and anxiety during threatening contexts

when there is something entirely unknown (i.e. not knowing anything)? As far as we are aware, no study to date has assessed this question directly.

Past empirical research has focused mainly on known uncertain threat (Tanovic, Gee, & Joormann, 2018). In these experiments participants are told about the threat contingencies. For example, participants are instructed: (1) that a given cue will result in a 50% chance of either receiving a negative or neutral picture (Grupe & Nitschke, 2011; Somerville et al., 2013), or (2) that there will be predictable or unpredictable countdowns to an electric shock (Nelson & Shankman, 2011). The findings for these studies are mixed with regards to IU. Some studies have found high IU to be associated with: (1) heightened amygdala activity to aversive pictures following unpredictable countdowns (Somerville et al., 2013), and (2) reduced startle blink magnitude during the anticipation of temporally uncertain threat (Nelson, Liu, Sarapas, & Shankman, 2016; Nelson & Shankman, 2011). Yet a number of these studies have not found significant relationships between IU and psychophysiological responses (Bennett, Dickmann, & Larson, 2018; Grupe & Nitschke, 2011; Morriss, 2019).

The closest empirical research has come to assessing unknown threat and IU may be in the associative learning literature (Chin, Nelson, Jackson & Hajcak, 2016; Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, 2015; Lucas, Luck, & Lipp, 2018; Morriss, Christakou, & Van Reekum, 2015, 2016; Morriss & Van Reekum, 2019). The majority of IU-related findings have been found during the extinction learning phase (Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, 2015; Lucas, Luck, & Lipp, 2018; Morriss, Christakou, & Van Reekum, 2015, 2016; Morriss, Morriss & Van Reekum, 2019). At the start of threat extinction learning the contingencies change and participants are not instructed about these changes, i.e. pairings between cues

(i.e. shapes, faces) and threatening stimuli (i.e. electric shocks or loud noises) are removed. The threat therefore becomes an unknown threat, as what was known is no longer applicable. During threat extinction learning, high IU is associated with continued skin conductance responding, corrugator supercilli activity (i.e. frowning) and amygdala activity to cues previously associated with threat (Morriss, 2019; Morriss, Christakou, & Van Reekum, 2015; Morriss, Saldarini, & Van Reekum, 2019). The absence of information regarding the change in threat and safe contingencies during extinction is thought to drive heightened responding in individuals with high IU (Dunsmoor, Campese, Ceceli, LeDoux, & Phelps, 2015; Morriss & van Reekum, 2019)

The disparity of IU-related findings in the different phases of associative learning (acquisition vs. extinction) suggest that under unknown conditions individuals with higher IU have a difficulty updating associations, rather than forming associations. Therefore, distress related to detecting or perceiving the unknown may be greater in situations where the contingencies were thought to be known i.e. expectations have been violated. It remains to be seen whether cues that signal the unknown influence psychophysiological responding in individuals with high IU in other contexts other than associative learning. Perhaps the heightened physiological responding observed in threat extinction for individuals with high IU is not specific but represents a more generalised behaviour related to detecting or perceiving the unknown.

Despite the emergence of IU research, there remains a gap in the literature on the extent to which known vs. unknown threat impacts psychophysiological markers of anxiety and fear in IU. Assessing how parameters such as uncertainty and unknowns influence psychophysiological responding in IU will help inform

current conceptualisations of IU and their relevance to psychopathology (Shihata, McEvoy, Mullan, & Carleton, 2016). Building upon previous research, here we examine how IU impacts physiological markers of fear and anxiety to known and unknown threat using a modified version of the threat of predictable and unpredictable aversive events task (Grillon et al., 2008; Grillon et al., 2009; Schmitz & Grillon, 2012). We used the standard instructed conditions of the threat of predictable and unpredictable aversive events task: known no shock, known unpredictable shock, and known predictable shock. In the known no shock condition, participants know they are safe from shock. Whilst in the known unpredictable shock condition participants know they can receive a shock at any time and in the known predictable shock condition participants know they will only receive a shock during a particular period of time. In addition, to these conditions, we presented an uninstructed condition to serve as the unknown threat. For the unknown threat condition participants did not receive any instructions and the cues changed from block to block. In the unknown threat condition no shocks were administered. Participants received the known conditions in a counterbalanced order first, before being exposed to the unknown threat condition. This ordering was used to maximise the distinction between the known and unknown conditions i.e. what participants think they know versus what they don't know.

During the modified threat of predictable and unpredictable aversive events task we presented auditory probes to elicit the startle blink reflex via the orbicularis oculi. The auditory probes were randomly presented during the cues (i.e. a shape) and interstimulus intervals (ISI). We assessed orbicularis oculi activity to the auditory probes during the cue and ISI. In addition, we assessed skin conductance response, pupil dilation and corrugator supercilii activity to the cues. Lastly, we collected self-

report ratings of valence and arousal at the end of each condition block. Throughout the modified threat of predictable and unpredictable aversive events task we recorded a multitude of self-report and physiological measures. The advantage of using a variety of measures is that they may reveal specific IU-related differences in arousal and valence (Lang, Bradley, & Cuthbert, 1990; Tassinary, Cacioppo, & Vanman, 2007). Skin conductance and pupil dilation are sensitive to measuring differences in arousal but cannot be used to measure differences in negative or positive valence (Beatty & Lucero-Wagoner, 2000; Dawson, Schell, & Filion, 2000). Whilst corrugator supercilli and orbicularis oculi activity are sensitive to measuring differences in arousal and valence, particularly aversive arousal (Lang et al., 1990; Tassinary et al., 2007).

We hypothesised that greater responding in skin conductance, corrugator supercilli activity, orbicularis oculi activity and self-report will be found for the known predictable shock condition, followed by known unpredictable shock, unknown threat, and known no shock conditions. This hypothesis is based on past research where greater responding has been found for conditions with predictable threat (Grillon et al., 2008; Grillon et al., 2009; Grupe & Nitschke, 2011; Morriss, 2019). Moreover, we expected that the unknown threat condition will be perceived as less aversive than the known predictable shock condition and known unpredictable shock conditions and more aversive than the known no shock condition because it is not associated with either threat or safety from shock.

If fear of the unknown is central to IU (Carleton, 2016b), then individuals with higher IU should display more self-report and physiological markers of fear and anxiety to cues signalling unknown threat during the modified threat of predictable and unpredictable aversive events task. Therefore, we hypothesised that higher IU,

relative to lower IU, would be associated with greater self-reported arousal/valence and responding in skin conductance and corrugator supercilii activity to the unknown threat, versus the known unpredictable shock, known predictable shock and known no shock conditions. Given that IU has been related to both increased/decreased orbicularis oculi activity during the known unpredictable shock condition (Nelson et al., 2016; Nelson & Shankman, 2011), we expected higher IU, relative to lower IU to be associated with increased/decreased orbicularis oculi activity to the probes presented during the cue and ISI for the known unpredictable shock versus the known predictable shock and known no shock conditions.

In line with our previous work (Morris, Christakou & van Reekum, 2016), the specificity of IU effects were assessed against trait anxiety. In this study we pitted IU against the Inventory for Cognitive and Somatic Anxiety (STICSA) (Ree, French, MacLeod, & Locke, 2008) because it is thought to be a purer measure of anxiety, compared to other trait anxiety measures which also feature depressive symptomology (Grös, Antony, Simms, & McCabe, 2007).

The current study protocol, power analyses and hypotheses were preregistered to the Open Science Framework and submitted as a registered report. The associated data and statistical output are available on the Open Science Framework (<https://osf.io/v2zgb/>).

Method

Participants

96 participants were recruited from the University of Reading and local area through advertisements and word of mouth (Sex: 49 Female, 44 Male; M age = 23.34 years,

SD = 4.47 years, range = 18 – 35 years; Ethnicity: 59 White, 21 Asian, 6 Mixed, 3 Middle Eastern/Arab, 3 Black, 1 Hispanic, and 3 not specified; Sexual Orientation: 78 Heterosexual, 13 Sexual Minorities (lesbian/gay/ bisexual), 5 not specified). For 3 participants there were technical errors (i.e. 2 participants did not receive any shocks during the task and 1 participant did not complete the task).

Participants were recruited if they were between 18-35 years of age and were not currently taking psychotropic medication. Participants were paid £10 to remunerate them for their time. The procedure was reviewed and accepted by the University of Reading Research Ethics Committee. The research was performed in accordance with relevant ethical guidelines/regulations and informed consent was obtained from all participants.

Multilevel models (MLM) were used to analyse experimental data, where IU and STICSA scores were entered into the analysis as continuous predictor variables. MLM's can account for missing cases and therefore are more powerful than repeated measures ANOVA's (Quené & Van den Bergh, 2004). However, due to the complexity of the approach, it is difficult to calculate power and estimate the sample size for MLM (Peugh, 2010). Therefore, appropriate sample sizes were estimated based upon power analyses using a repeated measures ANCOVA. The sample size of this study was based on a power analysis using the average effect size ($\eta^2 = 0.1634$) taken from Stimulus x IU or Stimulus x Time x IU interactions for skin conductance magnitude from five previous experiments (4/5 with significant effects of IU) (Morriss et al., 2015; Morriss, Christakou, & Van Reekum, 2016; Morriss & van Reekum, 2019). The following parameters were used: effect size $f = 0.44$ (converted from $\eta^2 = 0.1634$), α error probability = 0.05, Power ($1 - \beta$ error probability) = 0.95, number of covariates = 2 (IU, STICSA), numerator $df = 3$. The total sample size

suggested was $n = 93$. Data collection was terminated when the required sample size was met. In the case of participant withdrawal or errors in recording (i.e. equipment failure), replacement participants were recruited.

Procedure

When participants arrived at the laboratory, they were informed about the experimental procedures. Firstly, participants were seated in the testing booth and asked to complete and sign a consent form as an agreement to take part in the study. Secondly, participants completed the questionnaires on a computer. Thirdly, participants were asked to wash their hands in water without any soap and remove any eye-makeup. Then, electromyography sensors were attached to the left corrugator supercilli and orbicularis oculi, and skin conductance sensors were attached to the participants' left hand. Fourthly, a stimulator was attached to the little finger of the participants' left hand. The modified threat of predictable and unpredictable aversive events task was presented on a computer, whilst ratings and physiology were recorded. Participants were instructed to: (1) maintain attention to the task and to stay as still as possible, and (2) respond to the rating scales that followed the end of each block of trials, using the number keys on the keyboard. The experiment took approximately 60 minutes in total.

Shock calibration

The possibility of electric shock to the little finger of the left hand was used to induce anxiety. Electric shocks were delivered via ADInstruments PowerLab 26T Isolated Stimulator using a MLADDF30 stimulating bar electrode. The stimulation level was set by exposing participants to an electric stimulation of 1 mA (10 pulses at 50 Hz)

and increasing the current in steps of 0.5 mA, up to a maximum of 10 mA, until a suitable participant-specific threshold was found ($M = 5.47$ mA, $SD = 3.32$ mA). Participants were instructed that the electric shock should be uncomfortable but not painful.

Modified threat of predictable and unpredictable aversive events task

The threat of predictable and unpredictable aversive events task used in this experiment was a variant of the original developed by Grillon and colleagues (Schmitz & Grillon, 2012). First, participants completed a habituation phase, where 9 audio probes (40 ms, 103 dB white noise with near instantaneous rise time) were presented (this lasted for ~ 2 minutes). Second, participants completed the testing phase. In this phase there were four within-subject conditions: known no shock (KN condition), known predictable shock (KP condition), known unpredictable shock (KU condition) and unknown threat (UT condition). Each condition was presented 3 times in a fixed order (counterbalances as either KP,KN,KU,UT,KP,KN,KU,UT,KP,KN,KU,UT or KU,KN,KP,UT,KU,KN,KP,UT,KU,KN,KP,UT).

Each condition block consisted of 5 cue presentations that lasted 6 seconds and inter-stimulus interval's (ISI) that lasted 10-14 seconds. The duration of each condition block was set to 90 seconds. The entire duration of the modified threat of predictable and unpredictable aversive events task was 1080 seconds (18 minutes).

In each condition a different stimulus was presented on the screen during the cue: a diamond was displayed during the known predictable shock condition, a semicircle in the known unpredictable shock condition, and a cross for the known no shock condition. A star, a pentagon and a triangle were presented for the uncertain

threat condition. During the entire duration of the condition, at the top of the screen was displayed “NO SHOCK” for the known no shock condition, “SHOCK ONLY DURING GREY DIAMOND” for the known predictable shock condition, “SHOCK AT ANY TIME” for the known unpredictable shock condition and “...” for the uncertain threat condition.

In both the known predictable shock and known unpredictable shock conditions, some shocks were delivered, while in the other two conditions (known no shock and uncertain threat) no shocks were administered (see Figure 1). In the known predictable shock condition shocks were delivered in 2 out of the 5 cue presentations (6 s following cue onset – the shock co-terminated with the offset of the cue) while in the known unpredictable shock condition shocks were delivered in 2 out of the 5 ISI's (3-12 s following ISI onset). In the known unpredictable shock condition the timing of the shock onset and the ISI period were pseudo-randomised, in order to ensure that the shocks occurred within the specified ISI period and did not interfere with the audio probes presented i.e. if there was a short ISI period (i.e. 10 seconds) then the shock occurred within a specified ISI period (i.e. 3-7 seconds) away from the start and end of that specific ISI period. Altogether 12 shocks were delivered during the entire experiment.

In addition to the shocks, audio probes (same as the ones presented in the habituation phase) were also presented in this task in order to measure the startle reflex. Audio probes were presented in all conditions during both the stimulus presentation (1-5 s following the onset) and the ISI (3-12 s following the ISI onset). The onset of the audio probes were pseudo-randomised, to ensure that the audio probes occurred within the specified cue or ISI period of given trial within a block.

At the end of each condition block, participants were asked to rate:

(1) the valence and (2) arousal of the last block. The scales ranged from 1 (Valence: very negative; Arousal: calm) to 9 (Valence: very positive; Arousal: excited).

Questionnaires

To assess IU and trait anxiety, we administered the Intolerance of Uncertainty Scale (Freeston et al., 1994) and STICSA questionnaires (Ree, French, MacLeod, & Locke, 2008). The IU measure consists of 27 items that are rated on a 5-point Likert scale. The STICSA consists of 21 items that are rated on a 4-point Likert scale.

Rating data scoring

Arousal and valence rating data were reduced for each participant by calculating their average responses for each experimental condition (known predictable shock, known unpredictable shock, known no shock, and uncertain threat) using the E-Data Aid tool in E-Prime (Psychology Software Tools Ltd, Pittsburgh, PA).

Skin conductance acquisition and scoring

Identical to past research (Morriss et al., 2019; Morriss & van Reekum, 2019), physiological recordings were obtained using AD Instruments (AD Instruments Ltd, Chalgrove, Oxfordshire) hardware and software. Electrodermal activity was measured with dry MLT116F silver/silver chloride bipolar finger electrodes that were attached to the distal phalanges of the index and middle fingers of the non-dominant hand. A low constant-voltage AC excitation of 22 mVrms at 75 Hz was passed through the electrodes, which were connected to a ML116 GSR Amp, and converted to DC before being digitized and stored. A PowerLab 26T Unit Model was used to amplify the skin conductance signal, which was digitized through a 16-bit A/D

converter at 1000 Hz. The electrodermal signal was converted from volts to microSiemens using AD Instruments software (AD Instruments Ltd, Chalgrove, Oxfordshire, UK).

Skin conductance responses were marked for the cue using ADInstruments software (AD Instruments Ltd, Chalgrove, Oxfordshire, UK) and extracted using Matlab R2017a software (The MathWorks, Inc., Natick, Massachusetts, United States). Skin conductance responses were scored when there was an increase of skin conductance level exceeding 0.03 microSiemens (Dawson et al., 2000). The amplitude of each response was scored as the difference between the cue/ISI probe onset and the maximum deflection prior to the signal flattening out or decreasing. Skin conductance response onsets and respective peaks were counted if the skin conductance response onset was within 0.5-6 seconds following cue onset. Trials with no discernible skin conductance responses were scored as zero (51.1% of trials). Skin conductance response magnitudes were square root transformed to reduce skew and z-scored to control for interindividual differences in skin conductance responsiveness (Ben-Shakhar, 1985).

Skin conductance response magnitudes were calculated from remaining trials by averaging skin conductance response transformed values and zeros for each participant for the following conditions (known predictable shock, known unpredictable shock, known no shock, and uncertain threat). We defined non-responders as those with 10% or less skin conductance responses to all cues (Xia, Dymond, Lloyd, & Vervliet, 2017). Five participants met the criteria for non-responding and were excluded from the skin conductance response analyses, leaving 88 participants with usable skin conductance response data.

Pupil dilation acquisition and scoring

Eye movements were recorded using a Tobii TX300 eye-tracker with a sampling rate of 300 Hz (Tobii Technology, Inc., Stockholm, Sweden). The eyetracker was calibrated using a standard 9 point grid at the start of the experiment.

Pupil dilation was extracted using R software (R Core Team, 2014). Eye blinks were removed and then the data were averaged for each 1000 ms window following cue onset, resulting in six windows of 1000 ms each. These data were baseline corrected by subtracting 1000 ms preceding each cue onset from a blank screen. Pupil dilation data were z-scored to control for interindividual differences in pupil dilation size (Leuchs, Schneider, & Spoormaker, 2019). Trials were averaged per cue and second window for each participant resulting in the following conditions (known predictable shock, known unpredictable shock, known no shock, and uncertain threat).

Due to communication difficulties between E-Prime and Tobii Pro, we only collected data from participants who displayed an adequate eyetracking calibration, leaving 53 participants with useable pupil dilation data.

Electromyography acquisition and scoring

Facial electromyography measurements of the left corrugator supercilii and orbicularis oculi were obtained by using two pairs of 4 mm Ag/AgCl bipolar surface electrodes connected to the ML138 Bio Amp. The bipolar surface electrodes were approximately 15 mm apart. The reference electrode was a singular 8 mm Ag/AgCl electrode, placed upon the middle of the forehead, and connected to the ML138 Bio Amp. Before placing the electromyography sensors, the skin site was slightly abraded with isopropyl alcohol skin prep pads, to reduce skin impedance to an

acceptable level (below 20 k Ω). Electromyography was sampled at 1000 Hz. A high-pass filter of 20 Hz was applied to the raw electromyography online (Solnik, DeVita, Rider, Long, & Hortobágyi, 2008). The electromyography signal was root mean squared offline (Fridlund & Cacioppo, 1986).

Orbicularis oculi and corrugator supercilii activity were extracted using R software (R Core Team, 2014). Orbicularis oculi activity were quantified as the difference between the baseline (25 ms before stimulus presentation) and the peak of the electromyography signal in a window 20–120 ms after stimulus onset (Blumenthal et al., 2005). On trials with no discernible response (i.e. baseline value was larger than post probe value) a score of zero was assigned (0.23% of trials). The data were visually inspected for artefacts (i.e. blinks in the baseline or excessive noise, identified as electromyography values above 40 μ V in the baseline (Bradford, Kaye, & Curtin, 2014)). Trials with artefacts were removed from the analysis (3.75% of trials). For the orbicularis oculi, eyeblink response magnitude was calculated per cue and ISI for each participant for the following conditions (known predictable shock, known unpredictable shock, known no shock, and uncertain threat). Following this the data were z-scored to control for interindividual differences in orbicularis oculi and corrugator supercilii activity.

Corrugator supercilii activity were averaged for each 1000 ms window following cue onset, resulting in six windows of 1000 ms each. These data were baseline corrected by subtracting 2000 ms preceding each cue onset from a blank screen. For the corrugator supercilii trials were averaged per cue and second window for each participant resulting in the following conditions (known predictable shock, known unpredictable shock, known no shock, and uncertain threat).

Analyses

Analyses were conducted using the mixed procedure in SPSS 24.0 (SPSS, Inc; Chicago, Illinois). We conducted separate MLM's for ratings, skin conductance response magnitude, pupil dilation, orbicularis oculi and corrugator supercilii activity.

For ratings, skin conductance response magnitude, pupil dilation, orbicularis oculi and corrugator supercilii activity we entered Cue (known predictable shock, known unpredictable shock, known no shock, and uncertain threat) at level 1 and individual subjects at level 2. In addition, for the orbicularis oculi we entered ISI (known predictable shock, known unpredictable shock, known no shock, and uncertain threat) at level 1 and individual subjects at level 2. For pupil dilation and corrugator supercilii activity an additional factor of Second (1,2,3,4,5,6) was included in the MLM's. IU and STICSA were included as continuous predictor variables in all of the multilevel models.

In all models, we used a diagonal covariance matrix for level 1. Random effects included a random intercept for each individual subject, where a variance components covariance structure was used. Fixed effects included Cue, ISI and Second. We used a maximum likelihood estimator for the multilevel models. We corrected post-hoc tests for multiple comparisons using the Benjamini-Hochberg False Discovery Rate procedure (Benjamini & Hochberg, 1995). Acceptable values for multiple comparisons had to be below the following: valence ratings, arousal ratings, and skin conductance response, $p < .016$; pupil dilation, $p < .011$; corrugator supercilii and orbicularis oculi activity, $p < .022$.

In the MLM's a significant interaction with one variable (IU or STICSA) but not the other suggested specificity. Based on our prior work, we expected such specificity for IU, but we explored interactions with STICSA. Where a significant

interaction was observed with IU (or STICSA), we performed follow-up pairwise comparisons on the estimated marginal means of the relevant conditions estimated at specific IU values of + or -1 SD of mean IU, adjusted for STICSA. Similar analyses have been published elsewhere (Morriss, Macdonald, & van Reekum, 2016; Morriss & McSorley, 2019; Morriss, McSorley, & van Reekum, 2017).

We reported the internal consistency of the questionnaire, ratings and physiological measures (raw condition means) by using cronbach alpha tests.

There were no statistical outliers for any of the measures (i.e. 6 standard deviations from the mean).

Deviations from registration

During data collection we encountered communication difficulties between the Tobii eyetracker and E-Prime software. When the pupil could not be detected by the Tobii eyetracker, E-Prime crashed. We contacted Tobii and E-Prime but neither of them could provide us with a solution for this error. Therefore, we decided that if it was not possible to calibrate the pupil before the start of the experiment, we would run the task without the eyetracker. This led to having eyetracker data for 53 participants out of the 93 participants recruited.

Further posthoc analyses were conducted to examine whether there were differences in orbicularis oculi activity depending on when the auditory probe was presented (Cue, ISI) overall and in relation to IU. In the additional MLM for the orbicularis oculi we entered Stimulus (known predictable shock, known unpredictable shock, known no shock, and uncertain threat) and Type (Cue, ISI) at level 1 and individual subjects at level 2. Both IU and STICSA were entered as continuous

predictor variables. All other model parameters were the same as outlined above in the 'analyses' section.

At stage 2 of the registered report, the definitions of the fear of the unknown and intolerance of uncertainty were edited in the introduction section of the manuscript (for further detail please see supplementary material) and errors made at stage 1 relating to reporting of methodological details were corrected (timings of shock and audio probes, eyetracking device).

Results

For the descriptive statistics and internal consistency of the ratings and physiological measures please refer to Tables 1-3. The full statistics of interactions between IU and STICSA with other factors (Stimulus, Cue Type, Second) are presented in table 4.

Questionnaires

Distributions of the self-reported anxiety measures are presented in Figure 2 and internal consistency of the questionnaires are reported in Table 3: IU ($M = 64.05$, $SD = 18.04$, range = 36-112); STICSA ($M = 39.35$, $SD = 8.63$, range = 23-59). IU was positively significantly correlated with STICSA, $r(91) = .482$, $p < .001$.

Ratings

The known predictable shock and known unpredictable shock conditions were rated as more negative than the known no shock and uncertain threat conditions, $ps < .001$ [Stimulus: $F(3,96.099) = 39.242$, $p < .001$; see Table 1 and Figure 3]. The valence ratings for the known predictable shock vs. known unpredictable shock and

the known no shock vs. uncertain threat conditions did not significantly differ, $ps > .07$.

The known unpredictable shock condition was rated as the most arousing, compared to the known predictable shock, known no shock and uncertain threat conditions, $ps < .05$ [Stimulus: $F(3,107.769) = 66.981$, $p < .001$; see Table 1 and Figure 3]. The known predictable shock condition was rated as more arousing than the known no shock and uncertain threat conditions, $ps < .001$. The arousal ratings for the known no shock vs. uncertain threat condition did not significantly differ, $p = .101$.

No significant interactions were observed with IU or STICSA for the valence and arousal ratings (see Table 4).

Skin conductance response

As predicted, during the cue period of the task larger skin conductance response was found for the known predictable shock condition, compared to the known unpredictable shock, known no shock and uncertain threat conditions, $ps < .001$ [Stimulus: $F(3,193.967) = 32.366$, $p < .001$; see Table 1 and Figure 3]. No other significant differences in skin conductance response were observed between the known unpredictable shock, known no shock and uncertain threat conditions, $ps > .2$.

No significant interactions were observed with IU or STICSA for skin conductance response during the cue period (see Table 4).

Pupil dilation

During the cue period of the task larger pupil dilation was found for the known predictable shock condition, followed by the uncertain threat, known no shock and

known unpredictable shock conditions, $ps < .05$ [Stimulus: $F(3,581.750) = 57.823$, $p < .001$; see Table 1 and Figure 3]. Pupil dilation was smallest 2 second post cue and largest 6 second post cue, in line with the light reflex [Second: $F(5,388.645) = 75.832$, $p < .001$].

No significant interactions were found with IU or STICSA for pupil dilation during the cue period (see Table 4)..

Orbicularis oculi

During the cue period of the task larger orbicularis oculi activity was observed for the known predictable shock condition, followed by the known unpredictable shock, known no shock and uncertain threat conditions, $ps < .001$ [Stimulus: $F(3,283.726) = 152.710$, $p < .001$; see Table 2 and Figure 4]. In addition, during the ISI period of the task greater orbicularis oculi activity was found for the known unpredictable shock condition, followed by the known predictable shock, known no shock and uncertain threat conditions, $ps < .001$ [Stimulus: $F(3,191.513) = 64.226$, $p < .001$; see Table 2 and Figure 4]. For the cue and ISI period, orbicularis oculi activity did not significantly differ between the known no shock and uncertain threat conditions, $ps > .1$. No significant interactions were observed with IU or STICSA for the orbicularis oculi during the cue and ISI periods (see Table 4).

Post-hoc analyses of orbicularis oculi activity

Larger orbicularis oculi activity was found overall for the known predictable shock condition, followed by the known unpredictable shock, known no shock, and uncertain threat conditions, $ps < .001$ [Stimulus: $F(3,383.566) = 196.520$, $p < .001$; Stimulus]. Orbicularis oculi activity did not significantly differ between the known no

shock, and uncertain threat conditions overall, $p = .145$. In addition, larger orbicularis oculi activity was found for the cue period vs. the ISI period, $p < .001$ [Type: $F(1,661.014) = 94.630$, $p < .001$]. Furthermore, orbicularis oculi activity was the largest for the known predictable shock condition during the cue period, relative to the other conditions during the cue and ISI period [Stimulus x Type: $F(1383.566) = 42.870$, $p < .001$].

Lower IU, relative to higher IU was associated with greater orbicularis oculi activity to the cue period vs. the ISI period, $ps < .001$ [Type x IU: $F(1,661.014) = 6.119$, $p = .014$; See Figure 5A]. No other significant interactions were observed with IU or STICSA for the orbicularis oculi during the cue and ISI periods (see Table 4).

Corrugator supercilii

For the cue period of the task larger corrugator supercilii activity was observed for the known predictable shock condition, followed by the known no shock, uncertain threat and known unpredictable shock conditions, $ps < .02$ [Stimulus: $F(3,1029.273) = 20.676$, $p < .001$; Stimulus x Second: $F(15,342.892) = 2.057$, $p < .012$; see Table 1 and Figure 4]. Corrugator supercilii activity during the cue period did not significantly differ between the known no shock, and uncertain threat conditions, $p = .921$. Notably, this pattern above for the corrugator supercilii was exaggerated for individuals with higher IU, $ps < .001$, whilst there were no significant differences for the corrugator supercilii between conditions for individuals with lower IU, $ps > .2$ [Stimulus x IU: $F(3,1029.273) = 14.238$, $p < .001$; see Figure 5B].

Corrugator supercilii activity was smallest 5-6 seconds post cue [Second: $F(5,648.769) = 7.622, p < .001$]. No other significant interactions were found with IU or STICSA for the corrugator supercilii during the cue period (see Table 4).

Discussion

In the current study we examined how IU affects physiological markers of fear and anxiety to known and unknown threat using a modified version of the threat of predictable and unpredictable aversive events task (Grillon et al., 2008; Grillon et al., 2009; Schmitz & Grillon, 2012). Higher IU, relative to lower IU was associated with: (1) less discriminatory orbicularis oculi activity between cue and interstimulus interval periods across conditions, and (2) larger corrugator supercilii activity to the known predictable shock condition and smaller corrugator supercilii activity to the known unpredictable shock condition, compared to the other conditions. These findings provide evidence that IU-related biases manifest differently depending on the physiological marker of fear and anxiety and the type of known-unknown threat i.e. orbicularis oculi activity was related to generalisation across conditions, whilst corrugator supercilii activity reflected distress/relief during conditions with known threat. The findings from this study inform current conceptualisations of IU, as well as further our understanding IU in relation to anxiety and stress disorders.

Replicating past research, the known predictable shock condition, relative to the other conditions elicited greater skin conductance, pupil dilation, corrugator supercilii and orbicularis oculi activity during the cue periods of the task (Grillon, Baas, Lissek, Smith, & Milstein, 2004; Grillon et al., 2008; Grupe & Nitschke, 2011). In line with previous findings, the known unpredictable shock condition, relative to the other conditions, elicited greater orbicularis oculi activity during the interstimulus

interval periods of the task (Grillon et al., 2004; Grillon, Levenson, & Pine, 2007). Furthermore, for the uncertain threat vs. known unpredictable shock and known no shock conditions, we found larger pupil dilation during the cue periods of the task. This effect likely occurred because the cue always changed shape for the uncertain threat condition. However, the uncertain threat condition was comparable to the known no shock condition for all the other rating and physiological measures. There are two possible explanations as to why the uncertain threat condition was not arousing or aversive; (1) the design of the uncertain threat condition was not optimal (i.e. participants didn't experience a sense of the unknown during this condition) or, (2) the uncertain threat manipulation did work as intended, but the unknown may be less arousing or aversive when it co-occurs with known predictable and unpredictable threats.

Based on the hypothesis that fear of the unknown is central to IU (Carleton, 2016b), we predicted that higher IU would be associated with greater ratings and physiological responses to the uncertain threat condition, relative to the other conditions. However, IU was not associated with any differences in ratings or physiological responses to the uncertain threat condition, compared to the other conditions. Instead, we found that IU was specifically related, over STICSA, to physiological responses during the known conditions (see below for further discussion). Building upon Carleton's (2016a) definition of IU, these findings provide support that individuals higher in IU may attend to conditions where there is some certainty (i.e. known predictable threat), in order to reduce uncertainty. However, the null findings for IU in relation to the uncertain threat condition, suggests that further work is needed to examine whether IU is driven by fear of the unknown in a pure sense.

Based on past research (Nelson et al., 2016; Nelson & Shankman, 2011), we hypothesised that IU would be related to either increased/decreased orbicularis oculi activity (startle) to the auditory probes presented during the cue and ISI for the known unpredictable shock vs. known predictable shock and known no shock conditions. However, we did not find any evidence to support this. Interestingly, post-hoc analyses in this study revealed that higher IU, relative to lower IU was associated with less discriminatory orbicularis oculi activity between cue and interstimulus interval periods across conditions. This effect has two potential explanations: (1) individuals higher in IU, relative to lower in IU were less reactive in orbicularis oculi activity overall, or (2) individuals with higher IU, relative to lower IU were prone to generalising threat across cue and ISI periods, regardless of the type of known or unknown they faced. Whilst this effect was not hypothesised, the second explanation follows previous literature on the role of IU in threat generalisation. A number of studies have highlighted how individuals high in IU generalise threat to safe stimuli during uncertain contexts, indexed across multiple behavioural (i.e. risk, avoidance) and physiological measures (neural and skin conductance response) (Bauer et al., 2020; Hunt, Cooper, Hartnell, & Lissek, 2019; Morriss et al., 2015; Morriss, Christakou, et al., 2016; Morriss, Macdonald, et al., 2016; San Martín, Jacobs, & Vervliet, 2020). The orbicularis oculi and IU findings for this study compared to past work (Nelson et al., 2016; Nelson & Shankman, 2011) may have differed due to experimental design choices. Firstly, in the current study we used a reinforcement schedule of 33%, whilst Nelson et al.(2016, 2011) used a reinforcement schedule of 100%. Secondly, in our study we only presented the shock during the ISI period (similar to previous work (Schmitz & Grillon, 2012)) whilst Nelson et al. (2016, 2011) used scrambled countdowns and shock at any point

during the cue and ISI period for the unpredictable condition, which may have made this condition more uncertain.

During the cue period of the task individuals with higher IU displayed: (1) larger corrugator supercilii activity to the known predictable shock condition, relative to the other conditions, and (2) smaller corrugator supercilii activity to the known unpredictable shock condition, relative to the other conditions. Whilst individuals lower in IU displayed no differences in corrugator supercilii activity between conditions. The corrugator supercilii finding suggests that individuals high in IU, relative to low IU, were potentially more distressed/relieved during the cue period for the known predictable shock and unpredictable shock respectively. We can speculate that individuals high in IU may have been more engaged during the cue period for the known predictable and unpredictable shock conditions because they could form an expectation of whether a shock was likely to occur or not. Such engagement by individuals high in IU may reflect attempts to reduce any uncertainty related to the known contingencies i.e. checking whether the contingency information matched the expected outcome.

Notably, during the modified threat of predictable and unpredictable aversive events task IU was associated with the orbicularis oculi and corrugator supercilii differently. Both electromyography measures reflect negative affective state. The orbicularis oculi activity to acoustic startle probes is considered more specific to fear and anxiety, whilst corrugator supercilii is thought to measure many aspects of negative affect (i.e. fear, anxiety, distress, irritation, pain), and even effort (Tassinari et al., 2007). The generalised engagement of the orbicularis oculi for individuals high in IU suggests that this may be a relevant measure for examining both fear and anxiety in IU. In the present study, we cannot infer the specific type of negative affect

or effort, indexed by the corrugator supercilii, which may be related to IU during the modified threat of predictable and unpredictable aversive events task. Although, the corrugator supercilii has rarely been used as a measure in the threat of predictable and unpredictable aversive events task (Kaye, Bradford, & Curtin, 2016), the results here suggest that it may be a useful measure of negative affect and effort in individuals with high IU, and clinically relevant populations during the threat of predictable and unpredictable aversive events task and other similar tasks.

We did not find any significant relationships between individual differences in IU and the self-report ratings or arousal-based physiological measures (i.e. skin conductance, pupil dilation). Only a few studies have found relationships between IU and self-report ratings (Morris, Macdonald, et al., 2016). The lack of consistent patterns between physiological and self-report rating measures for IU may be due to the time between events and rating periods, where ratings are provided retrospectively. The absence of IU-related effects on skin conductance response and pupil are surprising, given that the majority of IU research, particularly the threat extinction learning literature, has found relationships with these measures (for review, see (Tanovic et al., 2018)). In the present experiment, however, most of the conditions were instructed and therefore little or no learning/updating of contingencies was required. Perhaps arousal-based physiological measures are more relevant for examining IU-related processes during uncertain threat contexts that require contingency updating.

The current study had a number of strengths including the adherence to open science principles; the design, hypotheses and analyses were pre-registered and the data and statistical output are available via the Open Science Framework (<https://osf.io/v2zgb/>). In addition, the study had a large sample size, it combines

self-report and a number of psychophysiology measures and uses creative manipulations across conditions to allow novel questions regarding IU to be addressed. There are also several shortcomings. Firstly, for the known conditions, there was still some unknowns i.e. participants did not receive detailed instructions about the reinforcement rate of the shocks. Moreover, some participants reported that they found the instruction for the known predictable shock condition ambiguous, as it was not clear whether they would always receive a shock or not during the cue. The use of a partial reinforcement rate was necessary to maintain the perception of known uncertainty and reduce habituation to the shock. However, future research examining IU using the threat of predictable and unpredictable aversive events task may benefit from using a wider spectrum of known-unknown i.e. providing all of the information, some information and no information. Secondly, many participants anecdotally reported that they found the audio probe more aversive than the shocks, perhaps due to having control over the shock intensity level but not the audio level of the probe. Thus, control over aversive stimuli may be an important aspect to assess in future experiments that use the threat of predictable and unpredictable aversive events task generally, and in relation to IU. Thirdly, whilst the sample were free of psychotropic medication, we did not screen for history of mental health disorders, which may have resulted in more variance in behavioural and physiological measurement (Grillon et al., 2009; Lissek et al., 2013). Lastly, further replication studies are needed to assess the robustness of IU-related effects in the threat of predictable and unpredictable aversive events task.

In conclusion, these findings provide evidence that IU-related biases may arise differently depending on the marker of fear and anxiety and the type of known-unknown threat. These findings further our conceptual understanding of IU, which

will be relevant for the development of treatment interventions that target IU-related symptomology (Carleton, 2016b; Shihata et al., 2016). Further research is needed to explore how individual differences in IU modulate different aspects of behaviour during unknown and known threat in healthy and clinical samples.

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Declaration of interest statement

The authors declare no conflict of interest.

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Figure Captions

Fig 1. Depiction of the modified threat of predictable and unpredictable aversive events task.

Fig 2. Histogram distributions of self-reported IU and STICSA. Count refers to the number of participants with a particular IU/STICSA score.

Fig 3. Pirate plots with highest density intervals for each condition by measure: (A) valence rating, (B) arousal rating, (C) SCR during cue period, and (D) pupil dilation during cue period. Both known predictable and unpredictable shock conditions were rated as more negative and arousing than the other conditions. During the cue period of the task there was greater skin conductance and pupil dilation to the known predictable shock condition, relative to the other conditions. In addition, during the cue period of the task there was greater pupil dilation to the unknown threat condition, compared to the known unpredictable shock and known no shock conditions. Valence and arousal ratings from 1-9, where 1 is negative/calm and 9 is positive/excited. Square root transformed and z-scored SCR magnitude (μS), skin conductance magnitude measured in microSiemens. Z-scored pupil dilation (Δmm), measured in delta millimetres. Note that the z-scoring was performed within-subjects across conditions, thus explaining the negative values.

Fig 4. Pirate plots with highest density intervals for each condition by measure: (A) orbicularis oculi activity to auditory startle probes during cue period, (B) orbicularis oculi activity to auditory startle probes during interstimulus interval period, (C) corrugator supercilii activity during cue period. For the orbicularis oculi, there was

greater activity during the cue period of the task to the known predictable shock condition, relative to the other conditions, as well as greater activity during the interstimulus interval period of the task to the known unpredictable shock condition, relative to the other conditions. Moreover, for the corrugator supercilia during the cue period of the task, there was greater activity to the known predictable shock condition, relative to the other conditions, and lesser activity to the known unpredictable shock condition, compared to the other conditions. Z-scored orbicularis oculi and corrugator supercilia activity (μV), measured in microVolts. Note that the z-scoring was performed within-subjects across conditions, thus explaining the negative values.

Fig 5. Bar plots of the estimated marginal means from the multilevel model estimated at ± 1 standard deviation from the mean IU (controlling for STICSA) during the modified threat of predictable and unpredictable aversive events task: (A) orbicularis oculi activity to auditory startle probes during cue and interstimulus periods, and (B) corrugator supercilia activity during cue period for each condition. Higher IU was associated with: (1) less discriminatory orbicularis oculi activity between cue and interstimulus interval periods across conditions, and (2) during the cue periods of the task larger corrugator supercilia activity to the known predictable shock condition and smaller corrugator supercilia activity to the known unpredictable condition, relative to the other conditions. Bar represent standard error. Z-scored orbicularis oculi and corrugator supercilia activity (μV), measured in microVolts. Note that the z-scoring was performed within-subjects across conditions, thus explaining the negative value.

Table 1. Descriptive statistics (mean, standard deviation and range) by measure and stimulus type

	Known No Shock	Known Predictable Shock	Known Unpredictable Shock	Uncertain Threat
Valence rating (1-9)	5.35	4.52	4.40	5.17
	1.50	1.49	1.58	1.43
	2/9	1.67/9	1.33/9	2/8.67
Arousal rating (1-9)	3.71	4.92	5.18	3.91
	1.60	1.64	1.71	1.67
	1/8.33	1/8.33	1/8.33	1/8.33
Square root transformed and z-scored SCR magnitude ($\sqrt{\mu\text{S}}$)	-0.12	0.30	-0.07	-0.11
	0.28	0.36	0.24	0.24
	-0.39/1.30	-0.39/1.30	-0.64/0.66	-0.54/0.66
Z-scored pupil dilation (Δmm)	-0.20	0.45	-0.33	0.07
	0.54	0.56	0.60	0.63
	-1.13/1.25	-0.84/1.54	-1.35/0.83	-1.30/1.17
Z-scored corrugator supercilii activity (μV)	-0.05	0.26	-0.18	-0.04
	0.62	0.75	0.64	0.64
	-1.47/1.31	-1.50/1.47	-1.35/1.35	-1.50/1.50

Note: Valence and arousal ratings from 1-9, where 1 is calm/negative and 9 is excited/positive. SCR magnitude ($\sqrt{\mu\text{S}}$), square root transformed and z-scored skin conductance magnitude measured in microSiemens. Z-scored pupil dilation (Δmm), measured in delta milimetres. Z-scored corrugator supercilii activity (μV), measured in microVolts.

Table 2. Descriptive statistics (mean, standard deviation and range) for the orbicularis oculi by stimulus type

	Cue				ISI			
	Known No Shock	Known Predictable Shock	Known Unpredictable Shock	Uncertain Threat	Known No Shock	Known Predictable Shock	Known Unpredictable Shock	Uncertain Threat
Z-scored orbicularis oculi activity (μV)	-0.18	0.66	0.10	-0.20	-0.26	0.03	0.15	-0.31
	0.24	0.37	0.27	0.23	0.22	0.29	0.31	0.24
	-0.64/0.47	-0.22/1.45	-.042/0.61	-0.68/0.44	-0.88/0.45	-0.61/0.60	-0.54/0.88	-0.78/0.57

Note: Z-scored orbicularis oculi activity (μV), measured in microVolts.

Table 3. Internal consistency scores for all the measurements

	IU	STICSA	Valence rating	Arousal rating	Skin conductance	Pupil dilation	Orbicularis oculi	Corrugator supercilii
Internal consistency score (cronbach's alpha)	0.93	0.85	0.92	0.91	0.89	0.97	0.99	0.89

Table 4. Interactions between IU/STICSA with other factors (Stimulus, Cue Type, Second) for each measure

	Stimulus x IU	Stimulus x STICSA	Cue Type x IU	Cue Type x STICSA	Stimulus x Cue Type x IU	Stimulus x Cue Type x STICSA,	Sec x IU	Sec x STICSA	Stimulus x Sec x IU	Stimulus x Sec x STICSA
Valence rating	$F(3,96.099) = 2.139, p = .100$	$F(3,96.099) = .659, p = .579$	-	-	-	-	-	-	-	-
Arousal rating	$F(3,107.769) = 0.944, p = .422$	$F(3,107.769) = 1.409, p = .244$	-	-	-	-	-	-	-	-
Skin Conductance	$F(3,193.967) = .121, p = .948$	$F(3,193.967) = 1.028, p = .381$	-	-	-	-	-	-	-	-
Pupil Dilation	$F(3, 581.75) = 1.455, p = .226$	$F(3, 581.75) = 2.277, p = .079$	-	-	-	-	$F(5,388.645) = .598, p = .702$	$F(5,388.645) = .070, p = .997$	$F(15, 203.338) = .478, p = .95$	$F(15, 203.338) = .229, p = .999$
Corrugator Supercilii	$F(3, 1029.273) = 14.238, p < .001$	$F(3, 1029.273) = 1.569, p = .195$	-	-	-	-	$F(5, 648.769) = .331, p = .894$	$F(5, 648.769) = .32, p = .901$	$F(15, 343.892) = .488, p = .946$	$F(15, 343.892) = .691, p = .793$
Orbicularis Oculi	$F(3, 383.566) = .551, p = .647$	$F(3, 383.566) = .583, p = .627$	$F(1, 661.014) = 6.119, p = .014$	$F(1, 661.014) = 2.528, p = .112$	$F(3, 383.566) = .973, p = .406$	$F(3, 383.566) = .558, p = .643$	-	-	-	-

KN	Known no shock condition
KP	Known predictable condition
KU	Known unpredictable condition
UT	Unknown threat condition
Audio probe Shock
Cues
UT Cues