Intolerance of uncertainty and physiological responses during instructed uncertain threat: a multi-lab investigation

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Jayne Morriss[1]*, Daniel E. Bradford[2], Shannon Wake[1], Nicolo Biagi[1], Ema Tanovic[3], Jesse T. Kaye[4,5] & Jutta Joormann[3]

[1]Centre for Integrative Neuroscience and Neurodynamics, School of Psychology and Clinical Language Sciences, University of Reading, Reading, U.K.

[2]Department of Psychology, University of Miami, Coral Gables, FL, USA

[3]Department of Psychology, Yale University, New Haven, CT, USA

[4]Center for Tobacco Research and Intervention, University of Wisconsin-Madison, School of Medicine and Public Health, Madison, WI, USA

[5]William S. Middleton Memorial Veterans Hospital, Madison, WI, USA

* Correspondence:

Jayne Morriss

j.e.morriss@reading.ac.uk

Abstract

Individuals with high self-reported Intolerance of uncertainty (IU) tend to interpret uncertainty negatively. Recent research has been inconclusive on evidence of an association between IU and physiological responses during instructed uncertain threat. To address this gap, we conducted secondary analyses of IU and physiology data recorded during instructed uncertain threat tasks from two lab sites (Wisconsin-Madison; *n* = 128; Yale, *n* = 95). No IU-related effects were observed for orbicularis oculi activity (auditory startle-reflex). Higher IU was associated with: (1) greater corrugator supercilii activity to predictable and unpredictable threat of shock, compared to the safety from shock, and (2) poorer discriminatory skin conductance response between the unpredictable threat of shock, relative to the safety from shock. These findings suggest that IU-related biases may be captured differently depending on the physiological measure during instructed uncertain threat. Implications of these findings for neurobiological models of uncertainty and anticipation in anxiety are discussed.

Keywords: Uncertainty, Threat, Instructed, Intolerance of Uncertainty, Corrugator Supercilii, Orbicularis Oculi, Skin Conductance

Introduction

Individuals who score high in self-reported Intolerance of Uncertainty (IU) tend to interpret uncertainty negatively (Carleton, 2016a, 2016b; Dugas, Buhr, & Ladouceur, 2004; Freeston, Rhéaume, Letarte, Dugas, & Ladouceur, 1994). Self-reported IU is a trait-based measure and is thought to capture a fundamental fear of the unknown (Carleton, 2016b). IU is described as '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). Importantly, IU is transdiagnostic and is observed in a number of mental health disorders that feature anxiety symptoms (Carleton, 2016b; Gentes & Ruscio, 2011; McEvoy & Mahoney, 2012). Research has begun to examine whether IU can be targeted in treatment for anxiety disorders and initial findings show promise (Dugas & Ladouceur, 2000; Mahoney & McEvoy, 2012; Oglesby, Allan, & Schmidt, 2017; van der Heiden, Muris, & van der Molen, 2012). On this basis the study of IU has gained substantial momentum over the last decade in the fields of anxiety research (Morriss, Zuj, & Mertens, 2021; Tanovic, Gee, & Joormann, 2018).

Despite advances in understanding IU, there still remain gaps in the literature on how IU modulates anticipatory physiological responses under different parameters of uncertainty (Morriss, Biagi, & Dodd, 2020; Morriss, Zuj, et al., 2021). In particular, a recent review (Tanovic, Gee, et al., 2018) highlighted that there were mixed findings for how IU modulates anticipatory physiological responses during instructed uncertain threat (typically also referred to as risk, irreducible uncertainty, first-order uncertainty and expected uncertainty (Angela & Dayan, 2005; Kobayashi & Hsu, 2017)). For example, during tasks where participants are instructed about the (un)predictability of aversive stimuli such as electric shocks or negative pictures,

some psychophysiology studies report IU-related effects (Gorka, Lieberman, Nelson, Sarapas, & Shankman, 2014; Morriss, Bell, Biagi, Johnstone, & van Reekum, 2021; Morriss, Bennett, & Larson, 2021; Morriss et al., 2020; Nelson, Liu, Sarapas, & Shankman, 2016; Nelson & Shankman, 2011; Somerville et al., 2013; Tanovic, Pruessner, & Joormann, 2018), while others do not (Grupe & Nitschke, 2011; MacNamara & Barley, 2018; Mertens & Morriss, 2021; Morriss, 2019; Morriss, Bell, et al., 2021). Furthermore, IU-related effects in instructed uncertain threat tasks vary considerably within and across physiological measures. For the orbicularis oculi (i.e. auditory startle-reflex), higher IU, relative to lower IU, is associated with: (1) potentiated and attenuated responses during unpredictable countdowns to shock (Nelson et al., 2016; Nelson & Shankman, 2011), (2) potentiated responses to safety from shock (Gorka et al., 2014; Morriss, Bennett, et al., 2021) and (3) poorer discriminatory responses between cue and interstimulus interval periods (Morriss et al., 2020). For the corrugator supercilii (i.e. facial frowning), higher IU, relative to lower IU is associated with greater responses during predictable shock, compared to unpredictable shock and safety from shock (Morriss et al., 2020). Lastly, for skin conductance, no IU related effects have been observed (Grupe & Nitschke, 2011; Morriss, 2019; Morriss, Bell, et al., 2021; Morriss et al., 2020).

The lack of consistent IU-related effects upon physiological measures during instructed uncertain threat tasks may be due to the degree of uncertainty in uncertain threat itself. For example, individuals high in IU may find instructed uncertain threat less aversive because the uncertain threat is ‘known’ and ‘reliable’ (for discussion see, Morriss, 2019). Additionally, it could be due to subtle differences in experimental design that alter the degree of uncertain threat (i.e. different aversive stimuli, different levels of (un)predictability, or varying number of conditions). However, what

is clear is that further empirical work is needed to examine the consistency of IU-related effects upon different types of physiological responses during instructed uncertain threat. Addressing this will allow us to assess the relevance of IU in instructed uncertain threat, as well as provide critical information for advancing our conceptual understanding of IU in relation to existing neurobiological models of uncertainty and anticipation (Brosschot, Verkuil, & Thayer, 2016; Grupe & Nitschke, 2013; Peters, McEwen, & Friston, 2017; Shihata, McEvoy, Mullan, & Carleton, 2016). Here, we conducted a secondary analyses of data (Kaye, Bradford, & Curtin, 2016a; Rutherford, Tanovic, Bradford, & Joormann, 2020) from two independent labs (Wisconsin-Madison, *n* = 128; Yale, *n* = 95) using instructed uncertain threat tasks to examine whether previously reported IU-related physiological profiles could be replicated.

Each lab site used a variant of the threat of predictable and unpredictable aversive events task (Grillon et al., 2008; Grillon et al., 2009; Schmitz & Grillon, 2012) with the following conditions: predictable threat of shock (i.e. shock always occurs during the cue period); unpredictable threat of shock (shock always occurs either in the cue or interstimulus interval period or shock occurs during the cue period with 20% reinforcement); and safety from shock (i.e. shock never occurs). Both lab sites recorded self-reported IU via commonly used questionnaires (Carleton, Gosselin, & Asmundson, 2010; Carleton, Norton, & Asmundson, 2007) and physiological responses: both lab sites measured orbicularis oculi activity (auditory startle-reflex) and corrugator supercilii activity (facial frowning), Yale recorded skin conductance response but neither lab had previously examined relationships between IU and physiological responses.

Based on prior research, we hypothesised:

H1. Higher IU would be associated with modulation of orbicularis oculi activity to unpredictable threat of shock, relative to predictable threat of shock and safety from shock (Nelson et al., 2016; Nelson & Shankman, 2011). Given that previous work has found inconsistent results for IU and orbicularis oculi activity, we did not hypothesise a particular direction of effect (i.e. potentiation or attenuation).

H2. Higher IU would be associated with greater orbicularis oculi activity to safety from shock (Gorka et al., 2014; Morriss, Bennett, et al., 2021).

H3. Higher IU would be associated with greater corrugator supercilii activity to predictable threat of shock, compared to unpredictable threat of shock and safety from shock (Morriss et al., 2020).

H4. No IU-related effects for skin conductance responding across any of the conditions (Grupe & Nitschke, 2011; Morriss, 2019; Morriss, Bell, et al., 2021; Morriss et al., 2020).

Following prior research (Morriss et al., 2020; Morriss et al., 2021; Nelson & Shankman, 2011), to assess specificity of IU, self-reported IU-related effects were assessed against other self-reported measures of broader negative affect from each lab site. The Wisconsin-Madison lab site used the Depression, Anxiety, and Stress Scales (DASS: Lovibond & Lovibond, 1995) and the Yale lab site used the State Trait Anxiety Inventory (STAI-T (the trait scale): Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983).

Method

Methodological details related to participants, procedure, task design and data collection are identical to that reported elsewhere (Kaye et al., 2016a; Rutherford et al., 2020). There is some overlap between the results reported here and that of

previous published work (i.e. main effects upon the orbicularis oculi activity and corrugator supercilii for the Wisconsin-Madison lab site and the orbicularis oculi activity for the Yale lab site). However, the current study uniquely assesses the relationship between multiple physiological variables (i.e. orbicularis oculi, corrugator supercilii and skin conductance) and intolerance of uncertainty, which have not been reported elsewhere. Furthermore, the physiological data were reprocessed across lab sites and different analysis approaches were used (i.e. multilevel models).

Participants

Wisconsin-Madison: A sample of one hundred twenty-eight participants from the undergraduate population and local community were recruited (64 female, 64 male; M_{age} = 23, SD_{age} = 7.7; 61% identified as White/Caucasian, 25% as Asian, 8% as Black/African American/African and 6% Other race; 5% of the sample identified as Hispanic/Latino).

The University of Wisconsin-Madison institutional review board approved the study and informed consent was obtained from each participant. Participants completed the experiment for course credit or $10/hour.

Yale: A sample of one-hundred and three participants were recruited from the community (62 female, 41 male; M_{age} = 32.13 years, SD_{age} = 12.43; 47% identified as White/Caucasian, 29% as Black/African American/African, 12% as Asian, 6% as more than one race and 3% as Latino/Latina). Out of one-hundred and three participants, ninety-five participants returned for session 2 (the session from which the task was taken from for the current study).

The Yale University Human Subjects Committee approved the study and informed consent was obtained from each participant before beginning the procedure. Participants were compensated $15.00 per hour.

Procedure

Wisconsin-Madison: Session 1 consisted of questionnaires, psychophysiological sensor application, shock calibration, a resting state task, the threat of predictable and unpredictable aversive events task and a picture viewing task. Session 2 consisted of the repetition of tasks from session 1. Data from the threat of predictable and unpredictable aversive events task from session 1 is reported in this study.

Yale: Session 1 consisted of questionnaires and a battery of other tasks (Tanovic, 2020). Session 2 consisted of psychophysiological sensor application, shock calibration, the threat probability task, and a battery of other questionnaires and tasks. Only data from the threat probability task is reported in this study.

Task design

Wisconsin-Madison: The threat of predictable and unpredictable aversive events task (Kaye et al., 2016a, Kaye et al., 2016b) was coded and presented via PsychToolBox (Brainard, 1997) in MATLAB (The MathWorks, Inc., Natick, Massachusetts, United States). Electric shocks were delivered using a custom built shockbox (Bradford, Magruder, Korhumel, & Curtin, 2014). The threat of predictable and unpredictable aversive events task comprised of three within-subject conditions: predictable threat (P), unpredictable threat (U) and no threat (N). All three conditions

(P, U, N) were presented in blocks of six trials in one of two fixed orders (PNUNUNP and UNPNPNU). Each threat condition (P, U) was presented twice and was separated by blocks of the no threat condition (N). Condition orders were counterbalanced across participants.

During each trial, “cues” were presented for 5 seconds with a variable ITI separating the cues (Mean ITI = 17 s, range = 14 – 20 s). In each trial, a coloured square “cue” was presented in the centre of the computer screen and indicated whether the participant would definitely receive a shock (P), possibly receive a shock (U) or never receive a shock (N). A white fixation cross remained in the centre of the computer screen during the presentation of the cue and ITI. During the predictable shock condition, a 200 ms shock was administered and always co-terminated with the trial. During the unpredictable threat condition, shocks were administered pseudo-randomly during the cues (at 2s or 4.8s post-cue onset) or during ITIs (at 4, 8 or 12 s post-cue offset). Participants received a total of 12 electric shocks in each of the predictable and unpredictable threat conditions. Shocks were never administered during the no-threat condition.

Before starting the task, participants were instructed of the cue-shock contingencies and were required to answer questions to confirm their understanding of the differences between the task conditions. Text was presented at the top of the screen (i.e. “Shock at End of Red Square”, “Shock at Any Time”, or “No Shocks”) for 9 s before the start of each block and remained on screen throughout the entire block. The shock electrode was disconnected prior to each no-shock block and was reconnected before each shock condition. Further, participants were required to provide a verbal response to the question “Can you be shocked in the next five seconds?” periodically throughout the task. Participants were asked to answer this

question (i.e., “yes” or “no”) whenever a question mark appeared on the screen in place of a fixation cross. Question marks appeared on screen four times during each shock condition and six times during the no shock condition. Data from participants who did not answer at least 10 out of 14 questions correctly were excluded from data analysis (n = 6).

Startle probes occurred at 4.5 s post-cue-onset on a random subset of 8 cues and 13, 14, or 15 s post-cue offset during 4 ITIs in both threat conditions. During no threat conditions, startle-probes were presented during 12 cues and 6 ITIs. Startle probes occurred a minimum of 12.5 s after another startle-eliciting event (e.g., shock or startle probe). Serial position of startle probes across the three conditions for both cues and ITIs was counterbalanced within-subjects to account for habituation. Two different orders of the serial position of the startle probe were used and were counterbalanced between-subjects.

Yale: The threat probability task (Bradford, Magruder, Korhumel, & Curtin, 2014; Rutherford et al., 2020) was coded and presented via PsychToolBox (Brainard, 1997) in MATLAB (The MathWorks, Inc., Natick, Massachusetts, United States). Electric shocks were delivered using a Grass Instruments stimulator (Grass Instruments, Quincy, MA, USA). The task consisted of three within-subject conditions: certain threat (P), uncertain threat (U) and no threat (N), with a total of 15 trials per condition. All three conditions were presented in six blocks of trials, and there were three counterbalanced orders in which blocks of trials could be presented (UNPPNU, NPUUPN, and PUNNUP).

During each trial, participants were presented with a “cue” that denoted the condition. Cues were presented for 5 seconds and were followed by a variable ITI

(range = 15 – 20 s). The cue consisted of a coloured shape (orange for certain, yellow for uncertain and green for no threat) and text stating the probability of receiving a shock (100%, 20% or 0%). During the certain threat condition, electric shocks were administered 100% of the time. During the uncertain threat condition, electric shocks were administered 20% of the time. Electric shocks were never administered during the no threat condition. In the certain and uncertain threat conditions, electric shocks were administered 4.5 s into the cue presentation and lasted 200 ms. Participants received a total number of 18 shocks throughout the task.

Before the task started, participants were instructed of the cue-shock contingencies and were asked to answer a series of questions to confirm their understanding of the differences between the task conditions. Before each block, text appeared on the screen for five seconds to inform the participant of what the next block would be *(e.g., "You are now entering a 100% SHOCK block.").* Only the text indicating the percentage of the probability of receiving a shock remained on the screen during the trial.

Acoustic startle probes (50ms bursts of 105dB white noise with near instantaneous rise time) were presented through headphones. Startle probes were delivered 4 s into the presentation of cues on a subset of trials (8 out of 15 trials in each condition). Probes were also delivered during the ITI (4 out of 15 ITIs in each condition; 13-15 s into the ITI) to decrease their predictability. Additionally, three startle probes were delivered at the start of the task, before any of the trials began to habituate the startle response before the main task. The order of blocks was counterbalanced and the serial position of probes was matched across conditions within subjects in order to balance the effects of habituation on the startle reflex.

Questionnaires

Wisconsin-Madison: The Intolerance of Uncertainty Index (IUI: Part A) (Carleton et al., 2010) and Depression, Anxiety and Stress Scales (DASS) (Lovibond & Lovibond, 1995) were collected. Higher scores represent greater IUI or DASS.

Yale: The 12-item short version of the Intolerance of Uncertainty Scale (IUS) (Carleton et al., 2007) and State-Trait Anxiety Inventory (STAI-Trait scale) (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983) were collected. Higher scores represent greater IUS or STAI-T.

Data collection of measures

Wisconsin-Madison: Participants were asked to wash their faces and hands with soap and water before the psychophysiology sensors were applied. The skin was further cleaned with alcohol swabs and exfoliated using an abrasive electrolytic gel before the electrodes were placed.

Data was recorded using Neuroscan bioamplifiers and Scan 4.5 acquisition software (Compumedics, Charlotte, NC) using a sampling rate of 2500 Hz with an online bandpass filter (1-500 Hz).

Startle eyeblink EMG activity was recorded from two 4-mm Ag-AgCl sensors placed beneath the right eye over the orbicularis oculi muscle. Corrugator response was recorded using two 4-mm Ag-AgCl sensors placed above the right eyebrow over the corrugator supercilii muscle. An 8 mm common ground sensor was placed in the centre of the forehead and a 4 mm reference sensor was placed 1 cm to the left. All

sensors were filled with conductive gel (ECI Electro-Gel; Electro-cap International, Eaton, OH).

Skin conductance response was not measured in this study.

Yale: Preparation procedures for psychophysiological measures followed those described for Wisconsin-Madison. The skin on the face was cleaned with alcohol swabs and exfoliated with an abrasive electrolytic gel, while the skin on the hands was washed with soap.

Data was recorded using a Neuroscan system and Curry acquisition software (Compumedics, Charlotte, NC) using a sampling rate of 5000 Hz. Pairs of Ag/AgCl electrodes were used for the recording of all data. Startle eyeblink EMG activity was recorded from two 4-mm Ag-AgCl sensors placed beneath the left eye over the orbicularis oculi muscle. Corrugator response was recorded using two 4-mm Ag-AgCl sensors placed above the left eyebrow over the corrugator supercilii muscle. Two 8-mm sensors placed at the top of the forehead served as the ground and reference. Further, skin conductance response was measured using two 8-mm Ag/AgCl electrodes placed on the thenar and hypothenar eminence of the non-dominant hand. All sensors were filled with conductive gel (SignaGel, Parker Laboratories Inc. Fairfield, NJ).

Reduction of orbicularis oculi activity

Wisconsin-Madison: Data was reduced offline using EEGLAB (Delorme & Makeig, 2004) and PhysBox plugins (Curtin, 2011) in MATLAB (The MathWorks, Inc., Natick, Massachusetts, United States). Offline processing included a high-pass filter (4th order 28 Hz Butterworth filter, zero phase shift), creating epochs from 50 ms pre-

probe to 250 ms post-probe onset, and signal rectification and smoothing (2nd order 30 Hz Butterworth low-pass filter, zero phase shift). Startle responses were quantified as the peak amplitude 20–100 ms post-startle probe onset relative to a 50 ms pre-probe baseline. Non-response trials for which the response amplitude did not exceed the maximum pre-probe amplitude were scored as 0.

An automated rejection algorithm was used to identify artefacts in each trial and reject trials from the analysis. Artefacts were classified as values greater than +/− 20 μV in the 50 ms pre-probe to 10 ms post-probe window (i.e. unstable baseline). The algorithm also identified an artefact if the mean amplitude during a trial was less than −10 μV in the 100–250 ms post-probe (i.e. movement artefact and baseline over-correction). Based on the aforementioned criteria, 0.3 – 1.1% of all trials in each task were rejected as artefacts. Trials were also examined manually and trials that were identified as atypical, based on the above criteria, and were not detected by the automated rejection algorithm, were manually rejected (0.5 – 1.2% of all trial in each task). Further, participants with general startle reactivity of < 5 μV during the Resting-State task were excluded as non-responders (n = 7), leaving a total of 121 participants with useable startle data.

Yale: The data reduction procedure for the orbicularis oculi was identical to that used by the Wisconsin-Madison lab site.

Participants who had more than 25% of trials within a given condition rejected were excluded from further analysis (startle response: n = 0). Participants were excluded because they declined to do the task (n = 2), the startle probes were presented at a volume of 75dB due to experimenter error (n = 3), the startle sensor

became loose (n = 1) and some had trouble staying awake (n = 4), thus leaving a total of 85 participants with useable startle data.

Reduction of corrugator supercilii activity

Wisconsin-Madison: Data processing was performed in Physbox via MATLAB (The MathWorks, Inc, Natick, Massachusetts, United States). Raw corrugator response was quantified in the time domain. Offline processing included a high-pass filter (4th order 28 Hz Butterworth filter, zero phase shift) and signal rectification. Epochs were created from 1000 ms pre-cue onset to 4500 ms post-cue onset. Trials were baseline corrected by subtracting mean activity 1000 ms pre-cue period from the entire cue period. Participants' trial level responses during the cue presentation (1000-4500 ms) were calculated and quantified as the mean amplitude in the participant's average waveform during the cue.

For corrugator analysis, trials were excluded when a startle probe occurred < 2 seconds pre-stimulus onset (0–4 trials per task) and when participants received a shock < 4.5 seconds post-cue onset (n = 2 trials in the unpredictable condition). Further, trials with deflections in the time domain greater than +/− 200 μV in raw signal across the entire epoch window were rejected as an artefact. Using these criteria, 1.5% of all trials as were excluded as artefact across tasks in both domains.

Participants with > 25% of trials rejected in the task were excluded from the analysis (n = 3). Further, participants with corrugator over > 200 μV or 60 Hz noise were excluded from corrugator analysis (n = 4). Further, participants who did not answer at least 10 out of 14 questions correctly were excluded from analyses (n = 6), leaving a total of 115 participants with useable corrugator data.

Yale: The data reduction procedure for the corrugator supercilii was identical to that used by the Wisconsin-Madison lab site, except for the length of the corrugator response window. Epochs were created from 1000 ms pre-cue onset to 4000 ms post-cue onset.

Participants who had more than 25% of trials within a given condition rejected were excluded from further analysis (n = 8). Additionally, participants were excluded because they declined to do the task (n = 2), startle probes were presented at a volume of 75dB due to experimenter error (n = 3), the startle sensor became loose (n = 1) and some had trouble staying awake (n = 4), thus leaving a total of 77 participants with useable corrugator data.

Reduction of skin conductance response

Yale: Analysis was performed using the Ledalab package (Benedek & Kaernbach, 2010) in MATLAB (The MathWorks, Inc., Natick, Massachusetts, United States). The data were downsampled to 50Hz and filtered using a Butterworth low-pass filter (order 1) with a low cutoff frequency of 5Hz. The signal was deconvolved using a continuous decomposition analysis that separates tonic and phasic activity. Phasic activity in response to task events was measured as the average phasic driver during the response window. Because skin conductance data is typically highly skewed, data were normalised using a logarithmic transformation.

Skin conductance responses were scored during the 1000 to 4000ms window after the onset of the trial.

Participants were excluded when the skin conductance was not recorded due to sensors not being correctly attached during the task (n = 6) or becoming loose during the task (n = 1). Additionally, participants were excluded because they

declined to do the task (n = 2), startle probes were presented at a volume of 75 dB due to experimenter error (n = 3), and some had trouble staying awake (n = 4), thus leaving a total of 79 participants with useable skin conductance data.

Physiological data normalisation

To control for individual differences in physiological responses due to skin type (i.e. dryness/oiliness of the skin), muscle size and variation in sensor placement, we z-scored data across trials for all physiological measures (Ben-Shakhar, 1985). The orbicularis oculi from the Wisconsin-Madison dataset was z-scored across cue and ISI periods. All other physiological measures from the Wisconsin-Madison and Yale datasets were z-scored across the cue period. Results from z-scored data are presented in the main text. Results from raw data are presented in the supplement for transparency, given controversy about standardization of psychophysiological measures such as the orbicularis oculi (Bradford, Starr, Shackman, & Curtin, 2015; Fridlund & Cacioppo, 1986). The majority of results were stable with regards to significance and direction across z-scored and raw data.

Analyses

Analyses were conducted using the mixed procedure in SPSS 25.0 (SPSS, Inc; Chicago, Illinois). To examine hypotheses 1-4, separate MLMs were conducted for each lab site and measure (i.e. orbicularis oculi; corrugator supercilii; SCR).

For the orbicularis oculi (Cue and ISI separately), corrugator supercilii and SCR, the following factors were entered: Stimulus (predictable threat of shock, unpredictable threat of shock, safety from shock) at level 1 and Subjects at level 2. Furthermore, to assess whether there are any differences in discriminatory

orbicularis oculi to the Cue and ISI period, an additional factor of Type (Cue, ISI) was entered at level 1 for the Wisconsin-Madison dataset. IUS/IUI was entered as a continuous predictor variable. Fixed effects included Stimulus, Type and random effects included Subjects. The MLMs included a diagonal covariance matrix for level 1 and a variance components covariance structure at level 2. For the MLMs, a maximum likelihood estimator was used.

If there was a statistically significant interaction with IUS and Cue/ISI, to test for specificity a further MLM was conducted including both IUS/IUI and STAI-T/DASS as continuous predictor variables. Follow-up pairwise comparisons consisted of the estimated marginal means of the relevant conditions at + or -1 S.D. of mean IUS/IUI, adjusted for STAI-T/DASS. The data at + or -1 S.D. of mean IUS/IUI, adjusted for STAI-T/DASS, is estimated from the multilevel model of the entire sample (Bauer et al., 2020; Morriss & McSorley, 2019) similar to performing a simple slopes analysis in a multiple regression analysis.

To further understand the direction of statistically significant interactions between IUS/IUI and physiological measures, we examined correlations (two-tailed) between IUS/IUI and each condition separately (i.e. predictable threat of shock, unpredictable threat of shock and safety from shock).

Results

Orbicularis Oculi

Wisconsin-Madison: For both the Cue and ISI periods, larger orbicularis oculi activity was observed for the unpredictable threat of shock condition, followed by the predictable threat of shock condition and safety from shock condition, *p*s < .002

[Cue: $F(2, 277) = 296.39$, $p < .001$; ISI: $F(2, 281) = 120.62$, $p < .001$; see Table 1 and Figure 1]. In addition, greater orbicularis oculi activity was observed for the Cue period, relative to the ISI period during the unpredictable and predictable threat of shock conditions, *p*s < .05. [Stimulus x Type: $F(2, 516) = 4.06$, $p = .018$). Furthermore, in general, larger orbicularis oculi activity was observed for the Cue, compared to ISI period [Type: $F(2, 547) = 9.56$, $p = .002$). No statistically significant interactions were observed with IUI for the orbicularis oculi, max $F = 0.53$ (see Figure 2).

Yale: During the Cue period, larger orbicularis oculi activity was observed for the unpredictable and predictable threat of shock conditions, relative to the and safety from shock condition, *p*s < .001. However, orbicularis oculi activity did not differ between the unpredictable and predictable threat of shock conditions, $p = .740$ [$F(2, 177) = 32.79$, $p < .001$; see Table 1 and Figure 1]. No statistically significant interactions were observed with IUS for the orbicularis oculi, max $F = 2.90$ (see Figure 2).

Corrugator Supercilii

Wisconsin-Madison: During the Cue period, greater corrugator supercilii activity was found for the unpredictable and predictable threat of shock conditions, relative to the safety from shock condition, *p*s < .001. Corrugator supercilii activity did not differ between the unpredictable and predictable threat of shock conditions, $p = .919$ [$F(2, 263) = 11.82$, $p < .001$; see Table 2 and Figure 3].

Individuals with high IUI demonstrated greater corrugator supercilii activity to both the unpredictable and predictable threat of shock conditions, relative to the

safety from shock condition, $p < .001$. All other pairwise comparisons for low and high IUI estimated from the MLM were non-significant, ps > .18 [Stimulus x IUI: $F(2, 263) = 5.43$, $p = .005$, see Figure 4; Stimulus x IUI (controlling for DASS): $F(2, 263) = 4.12$, $p = .017$]. Follow up correlational tests showed that higher IUI was negatively associated with corrugator supercilii activity to the safety from shock condition [$r(113) = -.24$, $p = .009$]. IUI was not significantly associated with corrugator supercilii activity to the unpredictable threat of shock condition [$r(113) = .07$, $p = .465$] or predictable threat of shock condition [$r(113) = .17$, $p = .065$]. The correlation between IUI and the safety from shock condition significantly differed from the correlation between IUI and the unpredictable threat of shock condition [$z = 2.36$, $p = .018$] and the correlation between IUI and the predictable threat of shock condition [$z = 3.15$, $p = .002$]. The correlations between IUI and the unpredictable and predictable threat of shock conditions did not significantly differ [$z = 0.79$, $p = .430$].

Yale: For the Cue period, larger corrugator supercilii activity was observed for the predictable threat of shock condition, relative to the unpredictable threat of shock and safety from shock conditions, ps < .01. However, corrugator supercilii activity did not differ between the unpredictable threat of shock and safety from shock conditions, $p = .816$ [$F(2, 152) = 4.62$, $p = .011$; see Table 2 and Figure 3]. No statistically significant interactions were observed with IUS for the corrugator supercilii, max $F = 0.50$ (see Figure 4).

Skin conductance response

Yale: During the Cue period, greater skin conductance response was found for the unpredictable, relative to the safety from shock condition, $p = .006$. Skin conductance

response did not differ between the unpredictable and predictable threat of shock conditions, p =.407, or between the safety from shock and predictable threat of shock conditions, p = .061 [F(2, 144) = 4.20, p = .017; see Table 3 and Figure 5].

Individuals with low IUS displayed greater skin conductance response to the unpredictable threat of shock condition, relative to the safety from shock condition, p < .001, whereas individuals with high IUS did not, p = .997. All other pairwise comparisons for low and high IUS estimated from the MLM were non-significant, ps > .057 [Stimulus x IUS: F(2, 144) = 4.20, p = .017, see Figure 6; Stimulus x IUS (controlling for STAI-T): F(2, 143) = 3.26, p = .041]. Follow up correlational tests demonstrated that higher IUS was negatively associated with skin conductance response to the unpredictable threat of shock condition [r(75) = -.26, p = .022]. IUS was not significantly associated with skin conductance response to the predictable threat of shock condition [r(75) = .03, p = .826] or safety from shock condition [r(75) = .19, p = .094]. The correlation between IUS and the unpredictable threat of shock condition significantly differed from the correlation between IUS and the safety from shock condition [z = 2.81, p = .005], but not the correlation between IUS and the predictable threat of shock condition [z = 1.78, p = .075].

Discussion

In the current study, we conducted secondary analyses of data from two independent labs (Kaye et al., 2016a; Rutherford et al., 2020) to examine the impact of IU upon different types of physiological responses during instructed uncertain threat. The experimental tasks used were based upon the threat of predictable and unpredictable aversive events task (Grillon et al., 2008; Grillon et al., 2009; Schmitz

& Grillon, 2012). No IU-related effects were observed for orbicularis oculi activity. Higher IU was associated with: (1) greater corrugator supercilii activity to the predictable and unpredictable threat of shock conditions, compared to the safety from shock condition and (2) poorer discriminatory skin conductance response between the unpredictable threat of shock condition, relative to the safety from shock condition. Importantly, these findings were specific to self-reported IU, over broader measures of negative affect such as the self-reported DASS and STAI-T. Such findings suggest that IU-related biases may be captured differently depending on the physiological measure during instructed uncertain threat. Taken together, these findings could advance our conceptual understanding of IU in instructed uncertain threat and have implications for existing neurobiological models of uncertainty and anticipation (Brosschot et al., 2016; Grupe & Nitschke, 2013; Peters et al., 2017).

We hypothesised that: (1) higher IU would be associated with modulation of orbicularis oculi activity to unpredictable threat of shock, relative to predictable threat of shock and safety from shock (Nelson et al., 2016; Nelson & Shankman, 2011) and (2) higher IU would be associated with greater orbicularis oculi activity to safety from shock (Gorka et al., 2014; Morriss, Bennett, et al., 2021). However, across both lab sites, we observed a lack of IU-related effects for the orbicularis oculi measure during the instructed uncertain threat tasks. Previous research has reported extensive variability in IU-related profiles for the orbicularis oculi measure during instructed uncertain threat tasks (Gorka et al., 2014; Morriss, Bennett, et al., 2021; Morriss et al., 2020; Nelson et al., 2016; Nelson & Shankman, 2011), including null effects (MacNamara & Barley, 2018; Mertens & Morriss, 2021). The inconsistent pattern of IU-related findings for the orbicularis oculi during instructed uncertain threat tasks suggests that IU-based modulation of the orbicularis oculi may be

particularly sensitive to sample type (Lang, McTeague, & Bradley, 2016; McTeague & Lang, 2012) and task design differences that alter the level of uncertainty and threat within these tasks. Currently, it is difficult to identify what aspects of the sample type and experimental design are responsible for IU-related differences upon the orbicularis oculi measure, given the variability of the sampling and experimental design across previous research using instructed uncertain threat tasks (i.e. different types of anxiety disorders [primarily panic disorder], reinforcement rate, the aversive stimulus content, timing of aversive stimulus and number of conditions). Further research parsing out these sampling and experimental design differences (Chin, Nelson, Jackson, & Hajcak, 2016; Morriss, Bennett, et al., 2021; Tanovic, Pruessner, et al., 2018) is required for understanding the role of IU in modulating the orbicularis oculi during instructed uncertain threat.

We hypothesised that higher IU would be associated with greater corrugator supercilii activity to predictable threat of shock, compared to unpredictable threat of shock and safety from shock (Morriss et al., 2020). We found partial evidence to support this hypothesis. For the Wisconsin-Madison lab site but not the Yale lab site, we found that higher IU was associated with greater corrugator supercilii activity to the predictable and unpredictable threat of shock conditions, compared to the safety from shock condition. This effect was driven by differences in IU to the safety from shock condition. More precisely, higher IU was associated with lower corrugator supercilii activity to the safety from shock condition. The difference in corrugator supercilii findings between the Wisconsin-Madison and Yale lab sites may be related to differences in reinforcement rate for the unpredictable condition (Wisconsin-Madison = 100%; Yale = 20%) and the extent to which shock reinforcement could occur in the cue or ISI period (Wisconsin-Madison = shock could occur in either the

cue or ISI; Yale = shock could only occur during the cue and not the ISI). The reinforcement rate and the extent to which shock reinforcement occurs in the cue or ISI period of the unpredictable condition likely changes the complexity and the perceived probabilistic structure of the experimental task overall. However, the lack of corrugator supercilii findings for the Yale lab site may also simply reflect a null result due to unaccounted for differences in sample characteristics or the lower N for that site. Notably, the finding from the Wisconsin-Madison lab site partially replicates prior research by Morriss et al. (2020), who used a very similar experimental design and observed that higher IU is associated with greater corrugator supercilii activity to the predictable threat of shock condition, compared to the safety from shock condition. The obvious differences in design between the Wisconsin-Madison lab site and the Morriss et al. (2020) study is the reinforcement rate used for both the predictable and unpredictable threat of shock conditions (Wisconsin-Madison = 100%; Morriss et al. (2020) = 33%) and the reinforcement during the cue and ISI periods for the unpredictable threat of shock condition (Wisconsin-Madison = reinforced in either the cue or ISI period; Morriss et al. (2020) reinforced during the ISI period only). The findings across both studies (Kaye et al., 2016a; Morriss et al., 2020) suggest that IU-related effects for the corrugator supercilii during: (1) the predictable threat of shock and safety from shock conditions are relatively robust despite different reinforcement rates within the predictable condition, and (2) the unpredictable threat of shock condition may be more sensitive to differences in reinforcement rate within this condition (i.e. if, when and where the shock occurs). In sum, these findings suggest that IU modulates valence-based measures related to broader negative affect such as the corrugator supercilii during instructed uncertain threat.

We hypothesised no IU-related effects for skin conductance responding across any of the conditions (Grupe & Nitschke, 2011; Morriss, 2019; Morriss, Bell, et al., 2021; Morriss et al., 2020). However, we found that higher IU was associated with poorer discriminatory skin conductance response between the unpredictable threat of shock condition, relative to the safety from shock condition. Follow up tests revealed that this effect was driven by differences in IU to the unpredictable threat of shock condition. More specifically, higher IU was associated with lower skin conductance response to the unpredictable threat of shock condition. This finding is at odds with previous literature showing no IU-related effects for skin conductance response during instructed uncertain threat tasks (Grupe & Nitschke, 2011; Mertens & Morriss, 2021; Morriss, 2019; Morriss, Bell, et al., 2021; Morriss et al., 2020). The pattern reported here may reflect IU-related generalisation of skin conductance responding across unpredictable and safety conditions. A similar pattern of IU-related generalisation of skin conductance has also been found for uninstructed uncertain threat tasks (i.e. fear generalisation experiments; Bauer et a., 2020; Morriss, Macdonald & van Reekum, 2016). However, this interpretation is speculative, given that the majority of prior research has reported no IU-related effects upon skin conductance response during instructed uncertain threat tasks. On this basis, further research is required to understand the role of IU in modulating non-valence arousal-based measures such as skin conductance response during instructed uncertain threat.

In the context of the broader literature, these findings tentatively suggest that during instructed uncertain threat (e.g. risk, expected uncertainty), IU may be more consistently involved in modulating valence-based measures that relate to broader negative affect (i.e. corrugator supercilii), rather than valence-based measures that

relate to anxiety or fear specifically (i.e. orbicularis oculi) or non-valence specific arousal-based measures (i.e. skin conductance) (Dawson, Schell, & Filion, 2000; Tassinary, Cacioppo, & Vanman, 2000). Generally, the level of uncertainty embedded within instructed uncertain threat tasks is low because the contingencies are 'known' and 'reliable', which likely makes the task less anxiety- or fear-provoking and arousing overall, even for individuals who score high in IU. Indeed, when the level of uncertainty is higher, such as in uninstructed threat tasks where the contingencies are 'unknown' or appear to be unreliable or volatile, effects of IU on physiological measures are more consistent (for review see, Morriss et al., 2021; Tanovic et al., 2018). The difficulty in mapping items or the total score from IU questionnaires to physiological responses during instructed uncertain threat tasks may be because the IU questionnaires themselves capture distress to more complex and abstract forms of uncertainty (e.g. focus on the future or in life generally) than would be observed in an instructed uncertain threat task based in a laboratory environment. Such discussion calls for further research to: (1) directly compare how IU impacts physiological measures under different parameters of uncertainty (e.g. Morriss et al., 2020; Morriss, Bennett & Larson, 2021), and (2) develop lab-based tasks that are more ecologically valid and reflect real life uncertainty. From the results of this study, it is also clear that extension of existing neurobiological models of uncertainty and anticipation (Brosschot et al. 2016; Grupe et al. 2013; Peters et al. 2017) is required, taking into account parameters of uncertainty (i.e. the level of uncertainty through parameters such as risk versus ambiguity) and its impact upon physiological responses.

This study had a few notable strengths. Firstly, the Wisconin-Madison and Yale datasets included several physiological measures and relatively large sample

sizes. Secondly, the datasets were reduced and analysed using the same procedures, thus improving their comparability. Thirdly, the specificity of IU-related effects could be compared with broader measures of negative affect such as the DASS/STAI-T. However, the study had some limitations. Firstly, the studies varied slightly in their experimental designs, limiting the extent to which particular experimental conditions could be compared. Secondly, the samples from the Wisconsin-Madison and Yale datasets mainly consisted of student and community participants. Although both datasets included participants with higher IU scores that were similar to that of observed in clinical samples (Carleton et al., 2012; Khawaja & Yu, 2010), the range was truncated towards the end of the scale. Therefore, the results from this study are not necessarily representative of clinical samples. Future research should address the extent to which these IU-related results in student/community samples are translatable to clinical samples (Gorka et al., 2014; Hiser, Schneider & Koenigs, 2021; Nelson et al., 2016), in order to further understand the relevance of IU as a transdiagnostic dimension in anxiety disorders (Carleton, 2016b; Shihata et al., 2016).

In conclusion, these findings suggest that IU-related biases may emerge differently depending on the physiological measure during instructed uncertain threat. These findings further our conceptual understanding of IU in relation to instructed uncertain threat (Shihata et al., 2016) and existing neurobiological models of anxiety and anticipation (Brosschot et al., 2016; Grupe & Nitschke, 2013; Peters et al., 2017). Further research is needed to explore how individual differences in IU modulate physiological responses during instructed uncertain threat, particularly in relation to different sample types (i.e. both dimensionally and categorically) and

different experimental design choices that alter the overall level of uncertainty and threat.

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Author contributions

E.T., J. J., D.B., and J.K. designed the experiments and collected the data. D.B., J.K., E.T., & N.B. organised the data for secondary analysis. J.M. wrote the introduction and discussion. J.M., S.W., E.T., J.J., D.B., and J.K. co-wrote the method. J.M. & N.B. conducted the analyses and co-wrote the results. E.T., J.J., D.B., and J.K. edited the final manuscript.

Declaration of interest statement

The authors declare no conflict of interest.

Figure Captions

Figure 1. Pirate plots with highest density intervals for orbicularis oculi activity to the auditory probes by condition. Data are presented for the cue period (Wisconsin-Madison: A; Yale: C) and interstimulus interval period (Wisconsin-Madison: B). Z-scored orbicularis oculi activity (μV), measured in microVolts.

Figure 2. For the Wisconsin-Madison (A-D) and Yale (E,F) datasets no statistically significant IU-related effects were found for the orbicularis oculi during the cue or interstimulus interval (ISI) periods. Correlations are presented for visualisation purposes only. Z-scored orbicularis oculi activity (μV), measured in microVolts.

Figure 3. Pirate plots with highest density intervals for the corrugator supercilli by condition during the cue period for the Wisconsin-Madison (A) and Yale (B) lab sites. Z-scored corrugator supercilii activity (μV), measured in microVolts.

Figure 4. Within the Wisconsin-Madison dataset, higher IU was associated with greater corrugator supercilii activity to the unpredictable (A) and predictable (B) threat of shock conditions, compared to the safety from shock condition during the cue period. For the Yale dataset, no statistically significant IU-related effects were found for the corrugator supercilii during the cue period (C, D). Correlations are presented for visualisation purposes only. Z-scored corrugator supercilii activity (μV), measured in microVolts.

Figure 5. Pirate plots with highest density intervals for skin conductance response by condition during the cue period for the Yale lab site. Z-scored skin conductance response (μS), measured in microSiemens.

Figure 6. For the Yale dataset higher IU was associated with poorer discriminatory skin conductance response between the unpredictable threat of shock condition, relative to the safety from shock condition during the cue period (A). No statistically significant IU-related effects were found for skin conductance response during the predictable threat of shock condition, relative to the safety from shock condition (B). Correlations are presented for visualisation purposes only. Z-scored skin conductance response (μS), measured in microSiemens.

Table 1. Means (Standard Deviations) for the orbicularis oculi by stimulus type.

Orbicularis Oculi						
	Z-Scored					
	Cue			ISI		
Lab Site	Unpredictable	Predictable	Safe	Unpredictable	Predictable	Safe
Wisconsin-Madison	0.37 (0.37)	0.21 (0.34)	-0.49 (0.25)	0.25 (0.51)	0.04 (0.54)	-0.48 (0.27)
Yale	0.12 (0.39)	0.11 (0.31)	-0.24 (0.33)			

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

Table 2 Means (Standard Deviations) for the corrugator supercilii by stimulus type.

Corrugator Supercilii			
	Zscored		
Lab Site	Unpredictable	Predictable	Safe
Wisconsin-Madison	0.06 (0.30)	0.06 (0.31)	-0.08 (0.22)
Yale	-0.03 (0.21)	0.06 (0.25)	-0.03 (0.24)

Note: Z-scored corrugator supercilii activity (μV), measured in microVolts.

Table 3 Means (Standard Deviations) for skin conductance response by stimulus type.

Skin Conductance Response			
	Zscored		
Lab Site	Unpredictable	Predictable	Safe
Yale	0.05 (0.25)	0.02 (0.27)	-0.07 (0.30)

Note: Z-scored skin conductance response (μS), measured in microSiemens.