PREDICTABLE AND UNPREDICTABLE THREAT REACTIVITY PROSPECTIVELY PREDICT INCREASES IN FEAR AND ANXIETY: AN ERP AND STARTLE INVESTIGATION

A Thesis

by

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ABSTRACT[1]

Fear (e.g., arousal, fight/flight) and anxiety (e.g., general distress, worry) are distinct dimensions of psychopathology that may underlie clinically significant variation in the internalizing disorders. Animal work supports this distinction as cues that predictably signal an upcoming aversive stimulus elicit fear behavior in rodents while uncertain threat cues activate more sustained, anxiety-like states. Moreover, this distinction between phasic fear and sustained anxiety is reflected in the NIH's Research Domain Criteria. The no-threat, predictable threat and unpredictable threat (NPU) task may probe core processes related to these dimensions. Prior work using this task has largely focused on categorical diagnoses. Knowing how predictable and unpredictable threat responding relate to transdiagnostic fear and anxiety may help identify and refine the underlying neurobiology. The purpose of this study is to assess associations between transdiagnostic fear and anxiety in the internalizing disorders and neurobiological response to predictable and unpredictable threat using the NPU-threat task. Furthermore, I will examine these associations prospectively to help inform prognostic trajectories for fear and anxiety, to facilitate potential early intervention or prevention efforts.

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1. INTRODUCTION[2]

In recent years, the NIH's Research Domain Criteria (RDoC; Insel et al., 2010), a project focused on developing a framework for investigating psychopathology that is dimensional and provides links to neurobiological systems, has emphasized identifying mechanisms underlying fear and anxiety dimensions. One domain within RDoC is negative valence system, which is responsible for responses to aversive events. Included in this system are the constructs of acute threat or fear (i.e., arousal or fight/flight response) and potential threat or anxiety (i.e., general distress or worry). These constructs can be viewed as basic defensive responses that motivate individuals/organisms to detect, react and cope with threats. Thus, these responses will vary depending on the presence of the threat, for example whether it is certain to occur would require immediate action or it is uncertain and instead would require a sustained state of vigilance (Schmitz & Grillon, 2012).

Animal work also supports organizing defensive behaviors into phasic fear versus sustained anxiety. For example, cues that predictably signal an upcoming aversive stimulus (e.g., shock) elicit short-term fear behaviors in rodents, whereas uncertain threat cues activate a more sustained, anxiety-like state (Davis et al., 2010). Recently, the theoretical distinction between fear and anxiety has been adapted into a transdiagnostic

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model for conceptualizing the anxiety disorders (Grillon et al., 2004; Hamm, 2020; Robinson et al., 2019). Transdiagnostic dimensions of fear and anxiety might provide a parsimonious way of conceptualizing the internalizing disorders and might be more faithful to underlying neurobiology than current, heterogeneous diagnostic categories. Here, I will test this theoretical framework by assessing associations between transdiagnostic fear and anxiety in the internalizing disorders and neurobiological response to predictable and unpredictable threat. More importantly, I also intend to assess whether predictable and unpredictable threat reactivity *prospectively* predict transdiagnostic fear and anxiety in order to determine if these constructs represent liabilities for the development of future psychopathology.

1.1. The No-Threat, Predictable Threat, and Unpredictable (NPU) Threat Task

The no-threat, predictable threat, and unpredictable (NPU) threat task was designed to probe predictable and unpredictable threat reactivity. Originally designed for use with eyeblink startle, the task involves three different trial types: no-threat (no aversive stimulus delivered), predictable threat (cues predict the delivery of an aversive stimulus) and unpredictable threat (aversive stimulus can be delivered at any time). The most common aversive stimulus employed in this task is shock. This task was originally designed for use with startle potentiation, and as such, startle probes are typically delivered during each of the cues and during the intertrial interval (ITI). Defensive reactivity to these startle probes is measured with electromyography (EMG), which captures the eyeblink component of the defensive reflex. Reactivity to predictable and unpredictable threat conditions is typically larger compared to the no-threat condition

(Nelson & Hajcak, 2017b). Increased startle responses during predictable threat cues in this task have been hypothesized to be associated with fear-based psychopathology, whereas increased startle reactivity during unpredictable threat cues is expected to be associated with anxiety (Grillon et al., 2004; Schmitz & Grillon, 2012).

1.2. Animal Work on Predictable and Unpredictable Threat

The distinction between fear and anxiety is evident at the neural level. For example, animal studies have shown that the bed nucleus of the stria terminalis (BNST), a region thought to influence behavior more in response to sustained symptoms of anxiety, might not be necessary for rapid-onset, short-duration behaviors in response to threat; instead it may only mediate slower-onset, longer-lasting responses that accompany more sustained threats (Walker et al., 2003). Moreover, lesions to the BNST have been found to blunt sustained anxiety states but did not affect phasic fear responses. Whereas when lesions were made to the central nucleus of the amygdala (CeA), a region thought to influence a more rapid response system to specific threat cues, was shown to blunt phasic fear responses to cues, without affecting sustained anxiety states (Walker et al., 2003). Similarly, Waddell and colleagues (2006) found that lesions to the BNST in rodents did not disrupt fear conditioning with shorter duration conditioned stimuli but they did attenuate responses to longer duration conditioned stimuli and reduce unconditioned anxiety in the rodents. Overall, these findings provide evidence from the animal literature that fear and anxiety are differentiated at the neural level.

1.3. Fear vs Anxiety in Internalizing Disorders

Despite the popularity of the NPU task (Grillon et al., 2017; Kaye et al., 2016; MacNamara & Barley, 2018; Radoman et al., 2019) and widespread acceptance of the fear versus anxiety model of anxiety (Gorka, Lieberman, Klumpp, et al., 2017; Grillon, Pine, et al., 2009; Radoman et al., 2019), few studies have explicitly tested hypothesized associations between fear and predictable threat responding and anxiety and unpredictable threat responding, particularly in a transdiagnostic fashion. Nonetheless, results to-date using the NPU task can be conceptualized within this framework of fear and anxiety when considering genetic and epidemiological data that has divided the internalizing disorders into: a) those characterized primarily by anxious misery/distress – e.g., generalized anxiety disorder (GAD), panic disorder (PD), agoraphobia, and post-traumatic stress disorder (PTSD); b) those characterized primarily by fear – e.g., the specific phobias (SP) and c) those that are somewhere in between – i.e., social anxiety (SAD; Chantarujikapong et al., 2001; Hettema et al., 2005; Kendler et al., 2003).

Most prior work using the NPU task in clinical samples has focused on PD, which, although dominated by worry about potential, uncertain threat (i.e., a future panic attack; Barlow, 2000), is also characterized by phasic fear (e.g., during panic attacks; Hamm, 2020; Robinson et al., 2019). This work has found that individuals with PD, (including those with other internalizing and externalizing comorbidities) have shown heightened startle response (i.e., defensive motivation) to unpredictable threat cues (Gorka et al., 2013; Gorka, Lieberman, Shankman, et al., 2017; Grillon et al., 2017; Lieberman et al., 2017). Startle responses to unpredictable threat cues have also been associated with a family history of PD (Nelson et al., 2013). Therefore, PD - and

potentially risk for PD - appears to be associated with unpredictable threat reactivity, in line with the notion that this disorder is characterized primarily by anticipatory anxiety about uncertain threat (panic attacks). Nonetheless, a few studies have found that individuals with PD, or elevated panic symptoms more generally, are characterized by increased startle potentiation to both unpredictable *and* predictable threat cues, in line with the notion that PD may be characterized by both phasic fear and sustained anxiety (Gorka et al., 2015; Shankman et al., 2013).

Neuroimaging work has, by contrast, suggested that PD is associated with hyperactivation across all threat and safety conditions, which is indicative of threat generalization, or decreased differential responding between conditions (Klahn et al., 2017). In addition, panic symptoms have been associated with increased reactivity to unpredictable threat cues in the dorsal anterior cingulate cortex (Lieberman et al., 2017) and in the brainstem (Radoman et al., 2019). Therefore, these findings support that anxiety symptoms, specifically those associated with panic, are associated with an increased reactivity to unpredictable threat. Additionally, panic susceptibility has been shown to predict dorsolateral prefrontal cortex (dlPFC) activity to unpredictable threat cues and this activity during unpredictable threat cues was associated with anxiety, suggesting that panic symptoms and anxiety are regulated by the same prefrontal cognitive control system (Balderston et al., 2017). Moreover, this association with the dlPFC, a regulatory region in the brain, suggests that individuals characterized by panic and anxiety are more sensitive to unpredictable threat cues requiring increased regulation in response to these cues. PTSD, which has typically been conceptualized as an anxiety

(not fear) disorder (Chantarujikapong et al., 2001; Scherrer et al., 2000), has been associated with increased startle response to unpredictable threat cues (Gorka et al., 2020; Gorka & Shankman, 2017; Grillon, Pine, et al., 2009). Taken together, these studies support an association between anxiety and unpredictable threat cue reactivity, as seen in both panic symptoms and in PTSD.

Fewer studies have examined predictable and unpredictable threat reactivity in disorders characterized primarily by fear. Research has shown mixed findings in individuals with SAD: some studies have found that SAD is associated with increased startle reactivity to predictable threat cues (Grillon et al., 2017), whereas others have found evidence of increased startle reactivity to unpredictable threat cues in SAD (Gorka, Lieberman, Shankman, et al., 2017). SP has also been associated with greater startle reactivity during unpredictable threat (Gorka, Lieberman, Shankman, et al., 2017; Nelson & Hajcak, 2017a), which is not in keeping with the hypothesized fear-predictable threat and anxiety-unpredictable threat associations. This work suggests that these disorders could be characterized to some degree by both fear and anxiety. Therefore, more work is needed to explore an association between fear and predictable threat reactivity specifically with a transdiagnostic approach, as opposed to categorical diagnoses, in order to parse out fear versus anxiety symptomatology, irrespective of heterogeneous diagnostic categories.

1.4. Prospective Association with the NPU-Threat Task

Use of the NPU task to examine prospective associations in the internalizing disorders is rare. One study found that cognitive behavioral therapy reduced startle

reactivity to unpredictable threat in individuals with PD, SAD, and PTSD (Gorka, Lieberman, Shankman, et al., 2017). Another study found that increased startle reactivity to both unpredictable and predictable threat cues predicted worse functional impairment at both baseline and approximately one year later in individuals with current and past internalizing disorders, controlling for diagnosis (Stevens et al., 2019). Specifically, these individuals had greater difficulty completing routine activities in their roles at home, work, school, or other social areas. Therefore, while limited, this work suggests that predictable and unpredictable threat reactivity might play a causal role in the internalizing disorders and might account for additional variance in outcomes beyond what can be explained by categorical diagnoses.

1.5. Categorical vs Transdiagnostic Considerations

Given the substantial heterogeneity known to characterize the current diagnostic categories (Insel et al., 2010), it would be ambitious to expect that hypothesized associations between disorders categorized as "fear" versus "anxiety" would show expected associations with predictable and unpredictable threat reactivity. That is, PD, SAD and SP may be characterized to some degree by both elevated fear and anxiety. Moreover, substantial comorbidity between categorical disorders means that several participants in these prior studies may have had other disorders, or anxiety psychopathology that did not reach threshold for diagnosis. For example, McTeague and colleagues (2011) demonstrated that in PD without agoraphobia, individuals exhibited the greatest startle reactivity to aversive images, but as comorbidity with agoraphobia increased from moderate to severe, startle reactivity to these images decreased,

suggesting that comorbidity could influence reactivity to threat cues. Transdiagnostic fear and anxiety are more homogeneous constructs than the current categorical diagnoses, and might be better suited to closing gaps with underlying neurobiology.

1.6. Stimulus-Preceding Negativity & the NPU-Threat Task

Electroencephalographic (EEG) event-related potential (ERP), the stimulus preceding negativity (SPN) is a negative-going slow wave component with a frontocentral distribution that grows larger (more negative) in the seconds prior to an anticipated event. The SPN has been shown to be larger in anticipation of emotional compared to neutral events (Grant et al., 2015) and is sensitive to stimulus probability (Catena et al., 2012; Lin et al., 2014). Links have been made between the SPN and activation in the dorsal and ventral attention systems (Brunia et al., 2011). Initial work has examined the SPN as a measure of threat anticipation during predictable and unpredictable threat cues (MacNamara & Barley, 2018; Tanovic & Joormann, 2019), MacNamara and Barley (2018) were the first to examine the SPN in the NPU-threat task and found that the SPN is larger (more negative) to threat (compared to no-threat) cues, though this effect was largely driven by larger SPNs during predictable cues. However, Tanovic and Joorman (2019) found that the SPN was larger in response to uncertain threat relative to certain threat within a community sample. In the current study, I will use the SPN to assess anticipation of predictable and unpredictable threat as it relates to continuous, transdiagnostic symptoms of fear and anxiety.

1.7. Current Study

The current study will examine the SPN, to assess threat anticipation at the electrocortical level, as well as startle eyeblink to assess defensive responding and to provide a link with prior work using the NPU-threat task, which has primarily used startle. Participants reported on here were a part of a larger study in which they were required to meet criteria for a focal fear disorder (defined as performance-only social anxiety or specific phobia), but could vary in levels of comorbid internalizing psychopathology. Therefore, this sample was well-suited to testing associations between fear, anxiety and predictable and unpredictable threat reactivity. I expect that individuals higher in transdiagnostic fear will show increased SPNs (i.e., threat anticipation) and heightened startle potentiation (i.e., defensive responding) to predictable threat cues, and that individuals higher in transdiagnostic anxiety will show increased SPNs and heighted startle potentiation to unpredictable threat cues (Davis et al., 2010; Grillon et al., 2004). I also expect to observe these associations *prospectively* – i.e., increased SPNs and startle to predictable threat cues at baseline will predict greater fear at follow-up 1.5 years later, and increased SPNs and startle to unpredictable threat cues at baseline will predict greater anxiety at follow-up, above and beyond baseline levels of fear and anxiety (Grillon et al., 2004; Robinson et al., 2019). If confirmed, these hypotheses would support the fear versus anxiety model of anxiety and would suggest that predictable and unpredictable threat reactivity might represent liabilities for the development of fear versus anxiety psychopathology, respectively. Moreover, if supported, this could provide potential prognostic indicators for advancing clinical care for individuals with fear and anxiety symptomatology.

2. METHODS[3]

2.1. Participants

All data used for this study was collected as part of a larger study. From this study, fifty-six individuals participated at both Time 1 (i.e., initial visit) and Time 2 (i.e., follow-up questionnaire approximately 1.5 years later); from this, four participants were excluded for having poor quality data recordings at one or both timepoints, leaving a final overall sample size of $n = 52$ (31 female; *M* age = 24.46 years, *SD* = 9.33). From this overall sample additional participants were excluded from individual analyses if they qualified as an outlier for the variables included (identified using Grubbs test; Grubbs, 1969). There were two outliers excluded for the late SPN leaving 50 participants for late SPN analyses (30 female; *M* age = 24.60 years, *SD* = 9.48). One outlier was excluded for startle, and six participants did not have a sufficient number of startle trials for analyses (≥2 trials), leaving 45 participants (26 female; *M* age = 23.49 years, *SD* = 7.78) for startle analyses.

Clinical characteristics of the sample at Time 1 and Time 2 are presented in Table 1. Although participants were recruited to fall into a psychiatrically healthy group (no current or prior psychiatric diagnoses; $n = 16$) or an anxiety/internalizing group based on the original larger study, my interest was in continuous associations across

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groups. All participants in the internalizing group (n = 36) were required to meet criteria for a focal fear diagnosis (specific phobia or performance-only social anxiety), but were permitted to vary in levels of additional, comorbid internalizing psychopathology (e.g., major/persistent depressive disorder [MDD/PDD], generalized anxiety disorder [GAD], generalized social anxiety disorder [SAD]). Exclusionary criteria for all participants included a history of a major medical or neurological illness, a history of traumatic brain injury, bipolar disorder, psychotic disorder, mental retardation, or developmental disorders. Diagnoses were made at Time 1 according to the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, DSM-5 (SCID; First et al., 2015). Participants were not engaged in psychiatric treatment of any kind (including no psychiatric medications within 6 weeks). Study procedures were in compliance with the Helsinki Declaration of 1975 (as revised in 1983), and were approved by the Texas A&M University institutional review board.

Table 1
Clinical characteristics of participants at Time 1 and Time 2

	Time 1	Time 2
	M (SD)	M (SD)
PANAS-X Fear	11.02 (4.26)	10.81 (4.59)
STAI trait	43.10 (12.76)	45.55 (11.39)
PSWQ	47.98 (16.24)	49.00 (14.23)
SPIN	18.98 (17.28)	20.10 (14.80)
# of current diagnoses	2.27 (1.93)	-
	n (%)	*n* (%)
Current Diagnosis		
Focal fear	36 (68)	-
SAD (Generalized)	19 (37)	-
GAD	11(21)	-
MDD/PDD	6 (12)	-
PTSD	4 (8)	-
PMDD	3 (6)	-
Agoraphobia	2 (4)	-
Anorexia nervosa	2 (4)	-
Substance use disorder	1 (2)	-

Note: PANAS-X, Positive and Negative Affect Schedule – Expanded Form; STAI, State Trait Anxiety Inventory; PSWQ, Penn State Worry Questionnaire; SPIN, Social Phobia Inventory; SAD, social anxiety disorder; GAD, generalized anxiety disorder; MDD, major depressive disorder; PDD, persistent depressive disorder; PTSD, posttraumatic stress disorder; PMDD, premenstrual dysphoric disorder. Focal fear included specific phobia (n=16) and performance-only social anxiety (n=20).

2.2. Materials

Dimensional psychopathology and internalizing symptoms were assessed using the Positive and Negative Affect Schedule-Expanded Form (PANAS-X; Watson & Clark, 1994), the Penn State Worry Questionnaire (PSWQ; Meyer et al., 1990), the State Trait Anxiety Inventory, trait version (STAI; Spielberger, 1983), and the Social Phobia Inventory (SPIN; Connor et al., 2000).

The PANAS-X (Watson & Clark, 1994) is a 60-item questionnaire that assesses ongoing positive and negative affect. Responses are made on a four-point Likert-type scale ranging from *very slightly or not at all* to *extremely*, with higher numbers indicating higher affect. Scores were computed for the Fear subscale.

The PSWQ (Meyer et al., 1990) is a 16-item questionnaire that assesses ongoing worry Responses are made on a five-point Likert-type scale ranging from *not at all typical of me* to *very typical of me*, with higher numbers indicating higher levels of worry.

The STAI (Spielberger, 1983), trait version, is a 20-item questionnaire that assesses ongoing anxiety. Responses are made on a four-point Likert-type scale ranging from *almost never* to *almost always*, with higher numbers indicating higher trait anxiety.

The SPIN (Connor et al., 2000) is a 15-item questionnaire that assesses social phobia symptoms over the past week. Responses are made on a five-point Likert-type scale ranging from *not at all* to *extremely*, with higher numbers indicating greater social phobia symptoms.

The PANAS-X Fear subscale was used as a continuous measure of transdiagnostic fear, computed separately at each timepoint (Time 1 Fear, Time 2 Fear).

Transdiagnostic anxiety was operationalized as a composite of averaged z-scored STAI, PSWQ, and SPIN scores, separately at each timepoint. This provided a broad measure of anxiety (Time 1 Anxiety, Time 2 Anxiety) that will not be specific to a particular diagnosis, in keeping with the characteristics of this mixed, internalizing, comorbid sample (Banica et al., 2020).

2.3. Procedure

2.3.1. Time 1

After consenting to participate in the experiment and completing questionnaires, participants' shock levels were set using standard procedures, in order to control for individual differences in shock sensitivity (Bradford et al., 2014; MacNamara & Barley, 2018). In brief, participants rated a series of increasing shocks to the wrist, using a scale from 0 (*can't feel shock*) to 100 (*highest you can tolerate*). Once they indicated that their level was at 100 no further shocks were administered and that level was used in the NPU-threat task.

While EEG was recorded, participants performed the NPU-threat task used in my lab's prior work (MacNamara & Barley, 2018) and adapted from Kaye and colleagues (2016), depicted in Figure 2.1. Participants were asked to view colored shape "cues" (blue circle, red square, green triangle) that were presented centrally on a computer screen. Each shape cue indicated whether the participant would definitely receive a shock (predictable or P), possibly receive a shock (unpredictable or U) or would never receive a shock (no-threat or N). Each condition was presented in a block of six trials; predictable threat and unpredictable threat blocks were interspersed with no-threat

blocks. Two condition block orders (PNUNUNP and UNPNPNU) were counterbalanced across participants. Cue pairings/assignment to condition was also counterbalanced across participants. On each trial, cues were presented for 5 sec with a variable intertrial interval (ITI) separating the cues (mean 17 sec, range 14-20 sec). During the cues and ITIs, a white fixation cross was presented in the center of the screen. In the predictable condition, a 200 ms shock was administered 200 ms prior to every cue offset (i.e., at 4.8 sec post-cue onset). In the unpredictable condition, shocks were administered pseudorandomly during cues (at 2 sec or 4.8 sec post-cue onset) or ITIs (at 4-12 seconds post-cue offset). In each threat block (P, U), participants received 6 electric shocks. No shocks were delivered in the N condition. Before beginning the real task, participants completed three practice trials to become familiar with the cues (no shocks were delivered during practice).

In order to ensure that the procedure and differences between the shock conditions were understood by the participants, they were first verbally instructed on the cue contingencies. Furthermore, reminders (e.g., “no shocks,” “shock at end of red square,” “shock at any time”) were displayed at the top of the computer screen for 9 sec prior to the beginning of each block and throughout the entire duration of the block (Kaye et al., 2016). Lastly, the shock electrode was removed from the participants’ wrists prior to the start of each no-threat block; it was then reapplied before the beginning of the next block.

Acoustic startle probes were delivered binaurally (40 ms, 90 dB white noise with near instantaneous rise time). Three initial startle probes were presented prior to the start

of the task to allow for stabilization of the startle response (Blumenthal et al., 2005); this data was not analyzed. Startle probes were presented at 4.5 sec post-cue onset on a pseudorandom subset of eight cues and at 13, 14, or 15 sec post-cue offset during four ITIs in predictable threat and unpredictable threat conditions (no-threat condition: startle probes during 12 cues and six ITIs). Each startle probe was presented a minimum of 12.5 sec after another startle-eliciting event (e.g., shock or startle probe). The serial position of startle probes across each condition was balanced within subjects to account for habituation. Two different orders of startle probe serial position were used and were counterbalanced between subjects.

2.3.2. Time 2

Just over a year and a half after their initial visit to the lab (M = 1.68 years; SD = 0.68), participants completed the same set of questionnaires completed at Time 1; due to the COVID-19 pandemic, questionnaires were completed online and participants did not complete the SCID or receive an EEG again at this time.

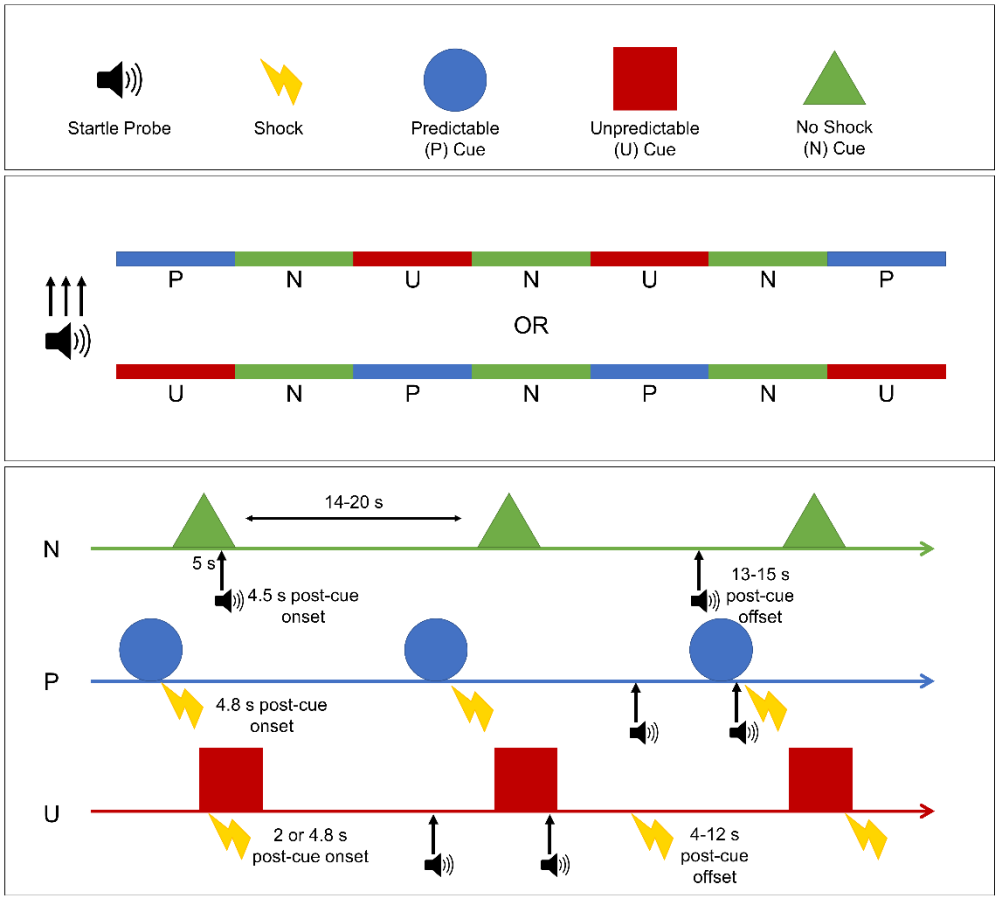

Figure 2.1 A depiction of sample trials from the no-threat, predictable threat, and unpredictable threat task.

2.4. EEG Data Acquisition and Data Reduction

Continuous EEG recordings were collected using an ActiCap and the ActiCHamp amplifier system (Brain Products GmbH, Gilching Germany) at Time 1. Thirty-two electrode sites were used based on the 10/20 system. The electrooculogram (EOG) was recorded from four facial electrodes: two that were placed approximately 1 cm above and below the right eye, forming a bipolar channel to measure vertical eye movement and blinks and two that were placed approximately 1 cm beyond the outer

edges of each eye, forming a bipolar channel to measure horizontal eye movements. The EEG data were digitized at 24-bit resolution and a sampling rate of 1000 Hz.

EEG data was processed offline using BrainVision Analyzer 2 software (Brain Products GmbH). Data was segmented for each trial beginning 200 ms prior to cue onset and lasting throughout the entire duration of the cue presentation (5200 ms in total); baseline correction for each trial was performed using the 200 ms pre-cue period. The signal from each electrode was re-referenced to the average of the left and right mastoids (TP9/10) and band-pass filtered with high-pass and low-pass filters of 0.01 and 30 Hz, respectively. Eyeblink and ocular corrections used the method developed by Miller, Gratton, & Yee (1988). Artifact analysis was used to identify a voltage step of more than 50.0 μV between sample points, a voltage difference of 300.0 μV within a trial, and a maximum voltage difference of less than 0.50 μV within 100-ms intervals. Trials were also be inspected visually for any remaining artifacts, and data from individual channels containing artifacts were rejected on a trial-to-trial basis.

Based on visual inspection of grand-averaged waveforms and topographic maps, the SPN was scored at Fz during an early window (1000-2000 ms post-cue onset) and a late window (3500-4500 ms post-cue onset; Morton et al., 2010); as in my lab's prior work (MacNamara & Barley, 2018), time windows were chosen to avoid shock delivery (which was at 2000 ms or 4800 ms on unpredictable trials and at 4800 ms on predictable trials) and startle probe delivery (at 4500 ms).

2.5. EMG Data Acquisition and Processing

Startle eyeblink EMG activity was recorded from two 4-mm diameter electrodes placed over the orbicularis oculi muscle under the left eye and using the ActiCHamp amplifier system. Data were digitized at 24-bit resolution and a sampling rate of 1000 Hz. EMG activity was band-pass filtered between 28-499 Hz and segmented using a 250-ms window that began 50 ms prior to startle probe onset. The data were rectified and were smoothed using a 50 Hz low-pass filter. Startle amplitude was quantified as the peak amplitude between 20 ms prior to startle probe onset and 150 ms after startle probe onset, relative to the average baseline (i.e., the average activity 50 ms prior to probe onset). Each trial was examined manually, and blinks were scored as nonresponses if EMG amplitude did not yield a peak that was visually differentiated from baseline activity; nonresponses were scored as 0. Blinks were determined to be missing if there was significant noise, movement artifact, or if a spontaneous blink was evident in the baseline period, because such factors can interfere with probe-elicited startle response (Blumenthal et al., 2005).

2.6. Data Analyses

To assess condition effects and group differences, cue-locked SPN amplitudes were submitted to separate 2 (group: control, anxious) X 3 (condition: no-threat, predictable threat, unpredictable threat) between-within analyses of variance (ANOVAs) and startle amplitudes were submitted to a 2 (group: control, anxious) X 2 (cue, ISI) X 3 (condition: no-threat, predictable threat, unpredictable threat) between-within ANOVA. Greenhouse-Geisser corrections were applied as necessary when the assumption of

sphericity was violated. Significant effects were followed up using dependent and independent samples *t*-tests, as appropriate.

To examine associations between psychophysiological measures and fear and anxiety at Time 1, we conducted separate linear regressions for each of the SPN and startle as predictors of Time 1 Fear and Time 1 Anxiety. For each regression, SPN/startle response to no-threat, predictable threat and unpredictable threat were entered as simultaneous predictors, along with the other Time 1 dimension (i.e., Fear or Anxiety).

To examine associations between psychophysiological measures and Time 2 symptoms, we conducted separate linear regressions for each of the SPN and startle elicited during no-threat, predictable threat and unpredictable threat, as predictors of Time 2 Fear and Time 2 Anxiety, controlling for Time 1 Fear and Time 1 Anxiety and the other experimental conditions (i.e., SPN/startle response to no-threat, predictable threat and/or unpredictable threat). In addition, because the time between Time 1 and Time 2 visits varied somewhat across participants, we also controlled for time passed between visits.

We used bootstrapped regression analyses (using 2000 bootstraps) which yielded bootstrapped *p* values and 95% confidence intervals (Westfall, 2011). Bootstrapping is a non-parametric resampling method that can produce more accurate Type 1 error rate and higher statistical power than the single sample parametric method (e.g., testing mediation effects; 48). Beta weights were considered significant when both the bootstrapped $p < 0.05$ and the confidence interval did not include zero (Grady et al.,

2015). Analyses of categorical diagnosis are presented in the Appendix. Analyses were performed using SPSS statistical software version 26.0 (IBM, Armonk, NY).

3. RESULTS[4]

Table 2 presents means and standard deviations for all psychophysiological measures, shown separately for each condition (no-threat, predictable threat, unpredictable threat). Table 3 presents regression results for both Time 1 and Time 2.

Table 2
ERP and startle means (standard deviations) for each condition

	No-threat (μV)	Predictable threat (μV)	Unpredictable threat (μV)
Early SPN	1.59 (5.93)	-1.63 (6.77)	2.00 (7.27)
Late SPN	1.87 (8.10)	-2.33 (9.22)	1.65 (11.29)
Cue Startle	49.86 (51.69)	82.08 (62.78)	89.06 (65.93)
ISI Startle	53.32 (55.84)	76.78 (71.22)	85.60 (74.85)

Note: ISI, interstimulus interval.

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Table 3

Regression results.

	Outcome: Time 1 Fear		
	Early SPN	Late SPN	Startle
N	0.011	0.040	-0.018
P	-0.004	-0.120*	0.009
U	-0.028	0.025	0.007
Time 1 Anxiety	3.490*	3.278*	3.542*
	Outcome: Time 1 Anxiety		
N	0.007	-0.001	0.001
P	-0.004	0.006	0.000
U	-0.001	-0.005	0.000
Time 1 Fear	0.153*	0.157*	0.173*
	Outcome: Time 2 Fear		
N	0.070	-0.022	-0.008
P	0.033	0.008	0.056*
U	-0.129	-0.035	-0.039
Time 1 Fear	0.700*	0.698*	0.830*
Time 1 Anxiety	0.163	0.315	-0.438
Time Passed (years)	0.004	0.003	0.003
	Outcome: Time 2 Anxiety		
N	-0.009	-0.018	0.001
P	0.023	0.011	0.008
U	-0.032*	-0.016*	-0.005
Time 1 Fear	0.040	0.050	0.044
Time 1 Anxiety	0.556*	0.533*	0.511*
Time Passed (years)	0.000	-0.000	0.000

Note: Columns represent separate regression models with analogous ERPs or startle entered as predictors of fear or anxiety. Regression coefficients are presented as bootstrapped, unstandardized beta weights. The SPN is a negative-going ERP component; therefore, negative beta-weights indicate that larger SPNs were associated with increased fear and/or anxiety. *bootstrapped $p < .05$

3.1. Time 1

Electrocortical activity

Early SPN. There was a significant effect of condition, $F(2, 100) = 4.45$, $p = .01$, $\eta_p^2 = .08$: predictable cues elicited larger (more negative) SPNs compared to no-threat cues, $t(51) = 2.64$, $p = .01$ and compared to unpredictable cues, $t(51) = 3.73$, $p < .001$. The early SPN to unpredictable and no-threat cues did not differ significantly, $p = .75$. The effect of group and the interaction between group X condition failed to reach significance, *p*s > .20. Dimensional analyses showed no significant associations between the early SPN to no-threat, predictable threat, or unpredictable threat and Time 1 Fear (*p*s > .58) or Time 1 Anxiety (*p*s > .69).

Late SPN. There was a significant effect of condition, $F(2, 96) = 3.56$, $p = .03$, $\eta_p^2 = .07$: predictable cues elicited larger (more negative) SPNs compared to no-threat cues, $t(49) = 2.90$, $p = .01$, and compared to unpredictable cues, $t(49) = 2.73$, $p = .01$. The late SPN to unpredictable and no-threat cues did not differ, $p = .91$. The effect of group and the interaction between group X condition failed to reach significance, *p*s > .06.

Dimensional analyses showed that larger late SPNs to predictable threat cues were associated with increased Time 1 Fear, $B = -.120$, CI: -.216, -.033, $p = .03$ (Figures 1 & 2). The late SPN to no-threat and unpredictable threat cues was not associated with Time 1 Fear, *p*s > .49. The other regression model, which aimed to predict Time 1

Anxiety found no association with the SPN to no-threat, predictable threat or unpredictable threat cues, *p*s > .62.

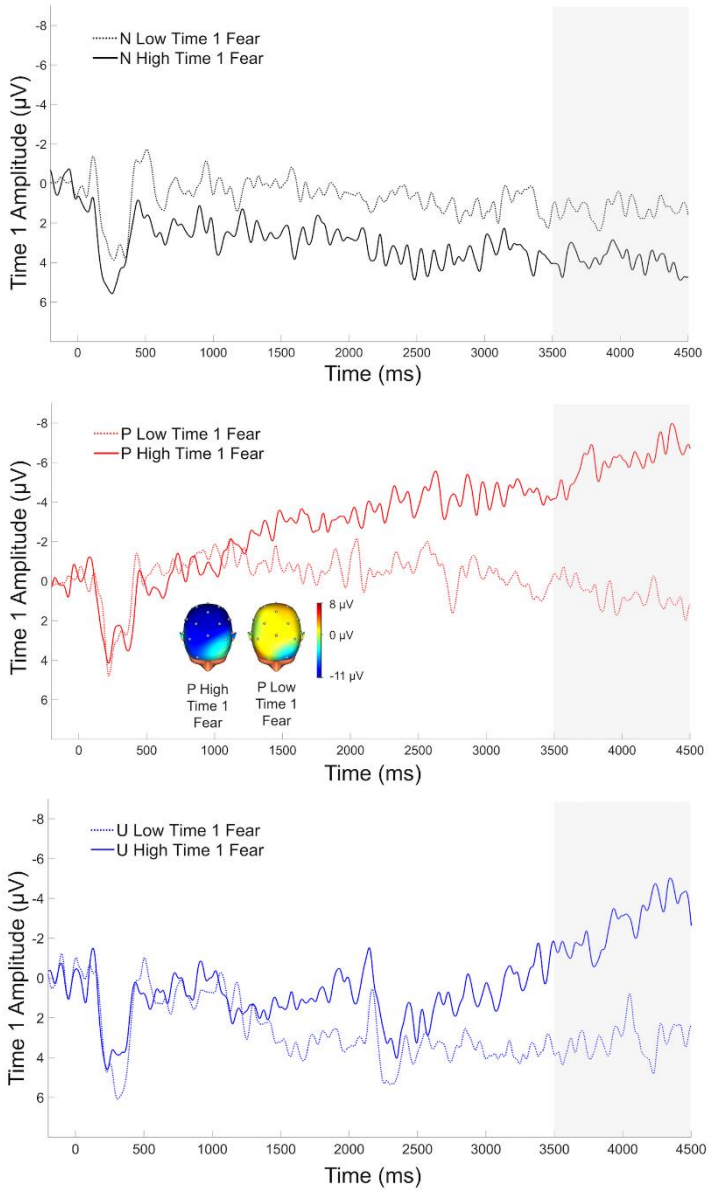

Figure 3.1 Time 1 late SPN to predictable threat and Time 1 Fear. Time 1 grand-averaged waveforms at Fz where the late SPN was scored, shown separately for no-threat (top), predictable threat (middle), and unpredictable threat (bottom), and for participants with high Time 1 Fear and low Time 1 Fear; positive is plotted downwards. Headmaps depict the voltage distributions for predictable threat cues, shown separately for participants with high Time 1 Fear and low Time 1 Fear.

Note: high Time 1 Fear (upper third) and low Time 1 Fear (lower third) groups were created for illustrative purposes only, all analyses were continuous.

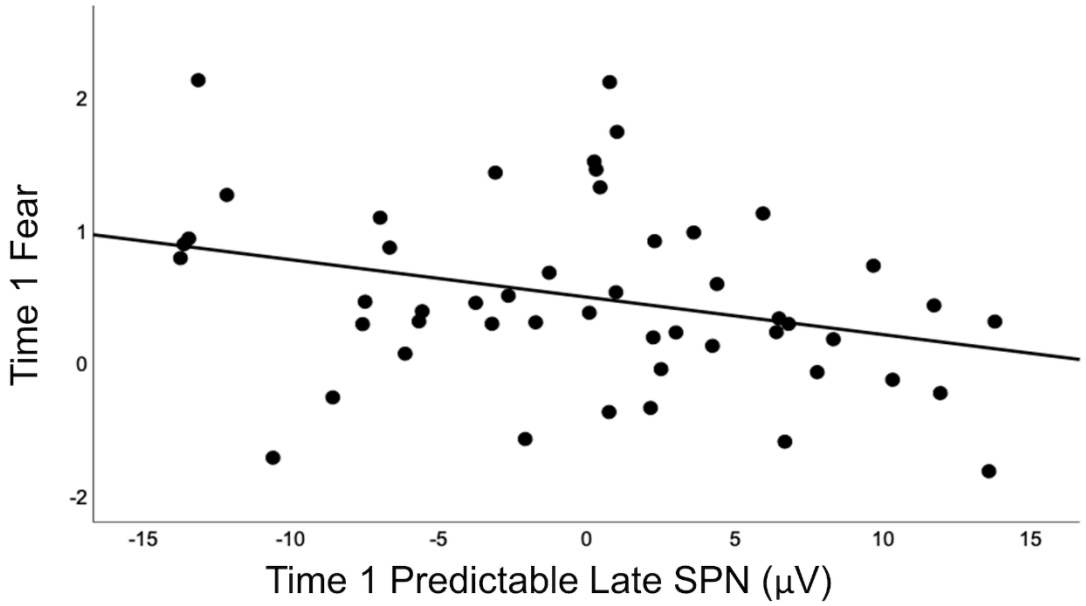

Figure 3.2 Scatterplot depicting the association (as unstandardized residuals after controlling for covariates) between Time 1 late SPN to predictable threat and Time 1 Fear.

Startle

There was a significant effect of condition, $F(2, 78) = 24.02$, $p < .001$, $\eta_p^2 = .38$: predictable threat (averaged across cue and ISI) elicited larger startle responses compared to no-threat, $t(44) = 5.46$, $p < .001$. Additionally, unpredictable threat elicited larger startle responses compared to no-threat, $t(44) = 6.33$, $p < .001$, and predictable threat, $t(44) = 2.68$, $p = .01$. No other effects reached significance at the omnibus level, ps > .13. Dimensional analyses showed no significant associations between startle to no-

threat, predictable threat, or unpredictable threat and continuous symptoms of Time 1 Fear (*p*s > .38) or Time 1 Anxiety (*p*s > .83).

3.2. Time 2

Electrocortical activity

Early SPN. Larger early SPNs to unpredictable threat cues predicted increased Time 2 Anxiety, *B* = -.032, CI: -.054, -.011, *p* = .01 (Figures 3 & 4). Early SPNs to no-threat and predictable threat cues were not associated with Time 2 Anxiety, *p*s > .06. The other regression model, which aimed to predict Time 2 Fear found no association with the SPN to no-threat, predictable threat or unpredictable threat cues, *p*s > .054.

Late SPN. Larger late SPNs to unpredictable threat cues predicted increased Time 2 Anxiety, *B* = -.016, CI: -.030, -.002, *p* = .04 (Figures 3 & 5). Late SPNs to no-threat and predictable threat cues were not associated with Time 2 Anxiety, *p*s > .15. The other regression model, which aimed to predict Time 2 Fear found no association with the SPN to no-threat, predictable threat, or unpredictable threat cues, *p*s > .41.

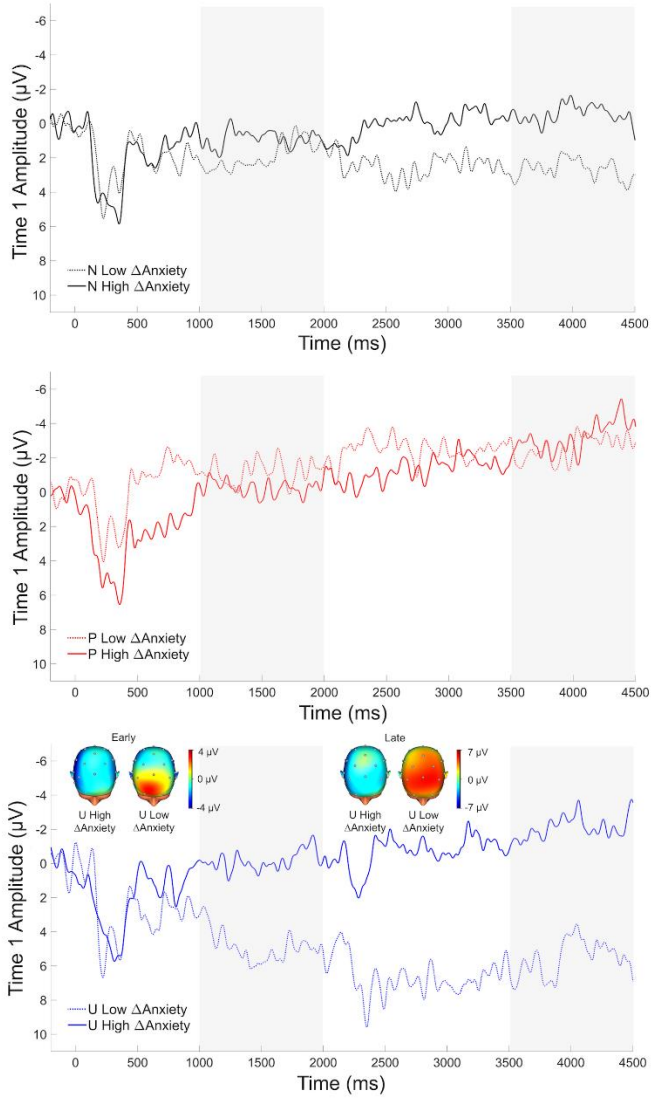

Figure 3.3 Time 1 early and late SPN to unpredictable threat and Time 2 Anxiety. Time 1 grand-averaged waveforms at Fz where the early and late SPN was scored, shown separately for no-threat (top), predictable threat (middle), and unpredictable threat (bottom) for participants with high ΔAnxiety and low ΔAnxiety; positive is plotted downwards. Headmaps depict the voltage distributions for unpredictable threat cues, shown separately for participants with high ΔAnxiety and low ΔAnxiety. Note: ΔAnxiety = Time 2 Anxiety – Time 1 Anxiety; high ΔAnxiety (upper third) and low ΔAnxiety (lower third) groups were created for illustrative purposes only, all analyses were continuous.

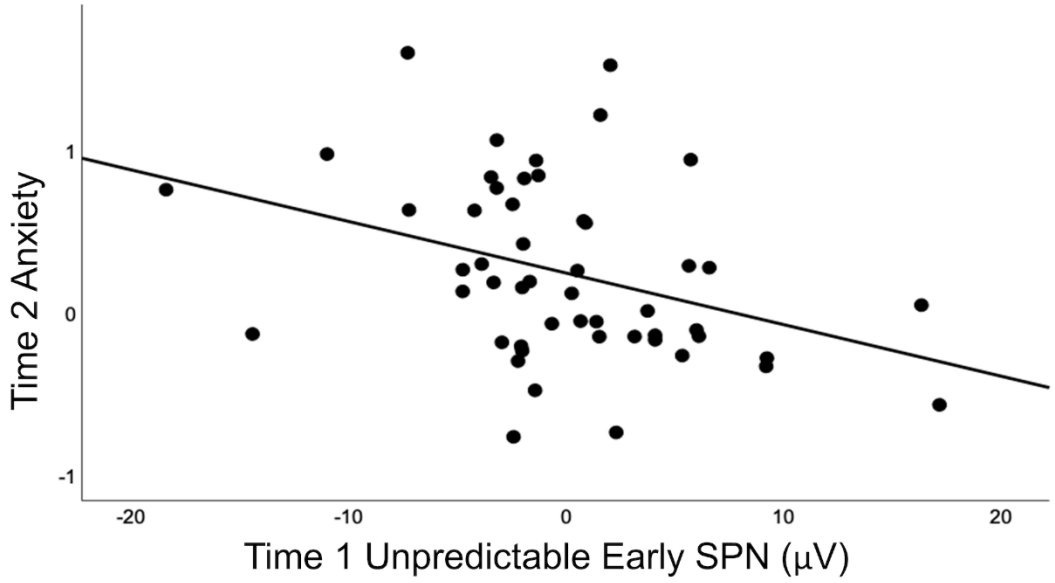

Figure 3.4 Scatterplot depicting the association (as unstandardized residuals after controlling for covariates) between Time 1 early SPN to unpredictable threat cues and Time 2 Anxiety.

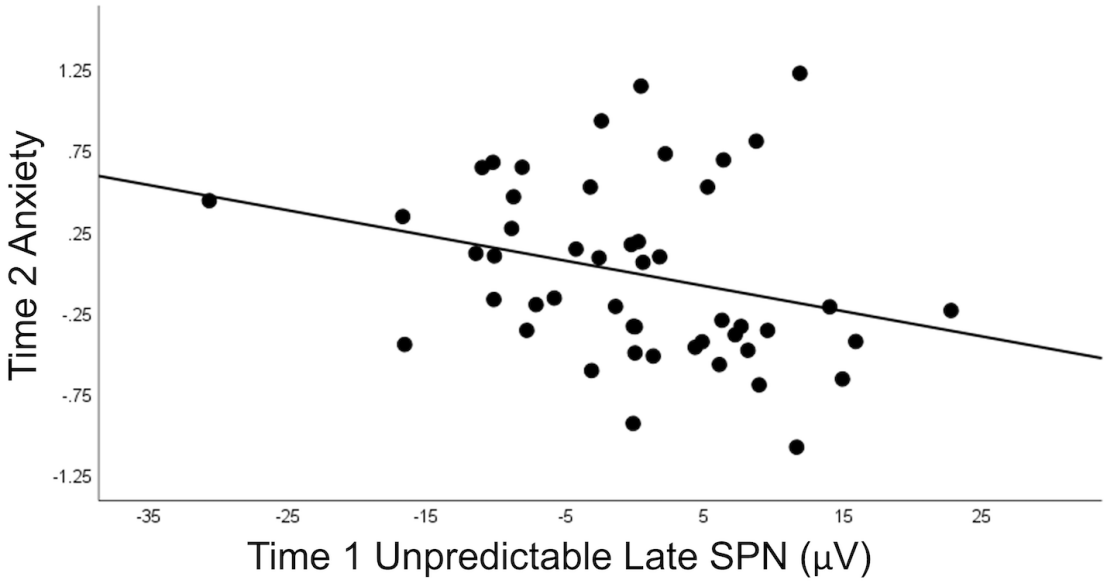

Figure 3.5 Scatterplot depicting the association (as unstandardized residuals after controlling for covariates) between the Time 1 late SPN to unpredictable threat cues and Time 2 Anxiety.

Startle

Larger startle responses to predictable threat predicted increased Time 2 Fear, *B* = .056, CI: .007, .098, *p* = .039 (Figure 6). Startle to no-threat and unpredictable threat was not associated with Time 2 Fear, *p*s > .054. The other regression model, which aimed to predict Time 2 Anxiety found no association with startle to no-threat, predictable threat or unpredictable threat, *p*s > .051.

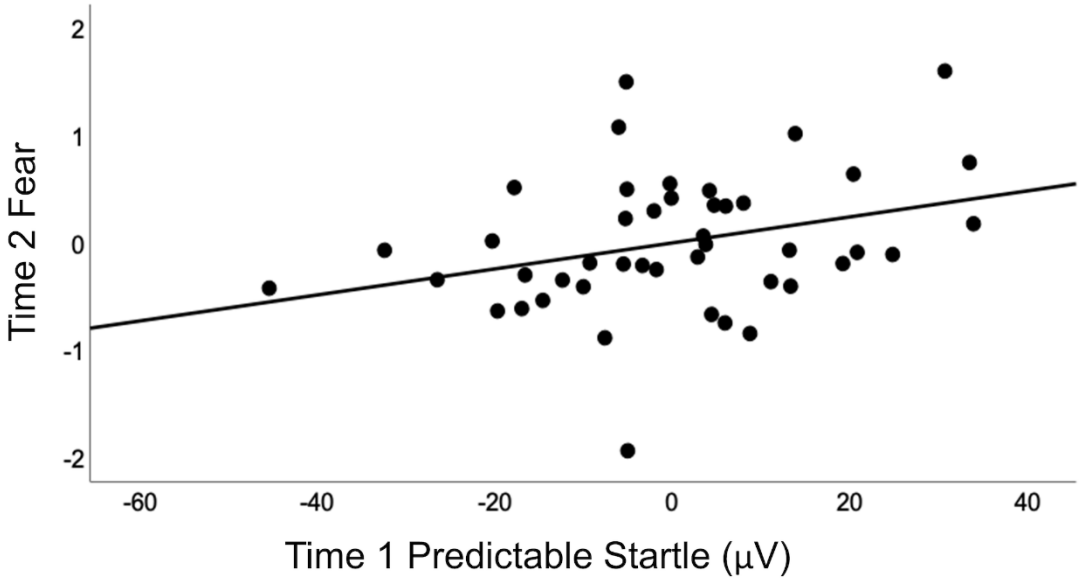

Figure 3.6 Scatterplot depicting the association (as unstandardized residuals after controlling for covariates) between Time 1 startle to predictable threat cues and Time 2 Fear.

4. DISCUSSION[5]

Heightened predictable and unpredictable threat reactivity have been hypothesized to underlie transdiagnostic fear and anxiety in the internalizing disorders, but these associations had not previously been tested. Here, we found that individuals with greater fear symptomatology at baseline were characterized by increased anticipation of predictable threat (SPN). Moreover, participants who showed greater defensive reactivity to predictable threat at baseline (startle) went on to show greater increases in fear symptomatology just over 1.5 years later. On the other hand, greater anticipation of unpredictable threat (SPN) at baseline uniquely and prospectively predicted increased anxiety. Results suggest mechanistic distinctions between transdiagnostic fear versus anxiety and implicate predictable and unpredictable threat reactivity as risk factors for the development of fear and anxiety psychopathology, respectively.

4.1. Cross-Sectional Transdiagnostic Fear & Predictable Threat

Prior work had failed to find evidence of an association between predictable threat reactivity and diagnosis of a quintessential fear disorder - specific phobia (SP). In fact, prior work examining SP found it was associated with greater startle reactivity during unpredictable threat (Gorka, Lieberman, Shankman, et al., 2017; Nelson &

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Hajcak, 2017a). As this is not in keeping with the hypothesized fear-predictable threat and anxiety-unpredictable threat associations, it suggests that this disorder may be characterized by both fear and anxiety. Nevertheless, our results do support the fear-predictable threat hypothesis as we found an association between increased anticipation to predictable threat cues and transdiagnostic fear symptoms. All else being equal, dimensional analyses offer more power than categorical analyses (Chmura Kraemer et al., 2004; Cohen, 1983); moreover, transdiagnostic fear is likely a more cohesive construct than categorical diagnosis of specific phobia. Therefore, dimensional assessment of fear – as in the current study - might more accurately "carve nature at its joints", and could explain why we observed an association between anticipation of predictable threat and transdiagnostic fear, where prior work had not.

4.2. Cross-Sectional Transdiagnostic Anxiety & Unpredictable Threat

We expected to find an association between transdiagnostic anxiety and greater defensive reactivity or sustained anticipation to unpredictable threat cues at Time 1, however, this hypothesis was not supported in the current study. Despite the majority of prior work supporting this hypothesis with evidence of an association between disorders predominantly characterized by anxiety being associated with increased defensive reactivity to unpredictable threat cues (Gorka et al., 2013; Gorka, Lieberman, Shankman, et al., 2017; Grillon et al., 2017; Lieberman et al., 2017), the current study failed to replicate this finding. Nevertheless, there is some prior evidence to support that anxiety disorders are still characterized to some degree by fear symptomatology (Gorka et al., 2015; Shankman et al., 2013). Thus, our lack of findings and this previous research

suggests that disorders may be characterized by symptoms of both anxiety and fear. Therefore, once you parse out the specific symptoms of anxiety and/or fear, this association is no longer significant. Moreover, this theory suggests that disorders such as PD, GAD, and PTSD may be characterized by more fear symptomatology than previously suggested from prior work.

4.3. Prospective Transdiagnostic Fear & Predictable Threat

When examining prospective associations, greater defensive reactivity (i.e., startle eye blink) to predictable threat cues predicted larger increases in fear symptoms over time. Proximal threat is associated with sudden increases in autonomic arousal (i.e., fight or flight response), thoughts of immediate danger (e.g., upcoming shock), and escape behaviors (Hamm, 2020; Robinson et al., 2019). Startle eye blink – a cross-species defensive response – may track the neurobiological pre-disposition to respond excessively to proximal threat, putting individuals at risk for greater fear symptomatology over time. While there is no prior prospective work that demonstrates an association between greater defensive reactivity to predictable threat and prospective transdiagnostic fear symptoms or the onset of a predominantly fear diagnosis, our results suggest that increased startle reactivity to certain threat might be a viable target for early intervention or prevention efforts aimed at reducing transdiagnostic fear.

4.4. Prospective Transdiagnostic Anxiety & Unpredictable Threat

In terms of prospective associations with anxiety, greater sustained anticipation (SPN) of unpredictable threat predicted larger increases in anxiety symptoms over time. These results are broadly in line with prior work, which had found evidence of cross-

sectional associations between anxiety and increased defensive responding to uncertain threat (Gorka et al., 2013; Gorka, Lieberman, Shankman, et al., 2017; Gorka et al., 2020; Grillon, Pine, et al., 2009; Grillon et al., 2017; Lieberman et al., 2017). Critically, results observed here suggest that heightened anticipation of uncertain threat may be present before the development and/or worsening of anxiety symptoms. Excessive anticipation of uncertain threat could underlie the development of behaviors such as avoidance, which can lead to increases in anxiety (Grupe & Nitschke, 2013). Therefore, early intervention or prevention efforts targeting anticipation of uncertain threat could be useful in combatting the development of or increases in transdiagnostic anxiety.

4.5. Broad Implications with RDoC & Animal Work

Both RDoC and animal work have supported a distinction between fear and anxiety symptomatology and predictable and unpredictable threat reactivity, respectively (Davis et al., 2010; Insel et al., 2010). RDoC identifies the domain of negative valence systems which includes acute threat or fear and potential threat or anxiety as basic defensive responses to threatening stimuli (Insel et al., 2010). Moreover, animal work demonstrate how cues that signal predictable threat or unpredictable threat consistently elicit short-term fear behaviors or more sustained, anxiety-like states, respectively (Davis et al., 2010). Our results align with RDoC's negative valence system model and with prior animal work, as we demonstrate associations between transdiagnostic fear and anxiety symptoms and predictable and unpredictable threat cue reactivity. Thus, our results support a distinction between fear and anxiety that are differentiated at the neural level in humans, expanding upon the differences seen in animal models.

4.6. Threat Anticipation vs. Defensive Responding

The unique associations we observed involving threat anticipation (SPN) versus defensive responding (startle) indicate that these measures provide different information about prospective fear versus anxiety psychopathology, with future fear best predicted by startle and future anxiety best predicted by the SPN. The SPN provides a measure of protracted and cognitively-mediated threat anticipation (Tanovic & Joormann, 2019). Therefore, our prospective results can be interpreted as indicating that sustained, future-oriented attention to the possibility of unpredictable threat is a risk factor for increased anxiety. This is in keeping with the notion that anxiety is characterized by heightened assessment of the probability and extent of threatening events (Grupe & Nitschke, 2013). Moreover, our results are in line with prior work, which found that among individuals at risk for future anxiety, excessive attention to threat over a period lasting several seconds was uniquely predictive of increased anxiety one year later (Bardeen & Daniel, 2018). In contrast, startle is a subcortically mediated measure of reflexive responding to threat (Kuhn et al., 2019). Therefore, our finding that increases in Time 2 Fear were predicted by greater startle response at baseline suggests that increased "bottom up" response to fear-provoking stimuli and/or failure to inhibit bottom-up responding might serve as a risk factor for the development of fear-based psychopathology (Feng et al., 2022; Peng et al., 2022).

Our cross-sectional results suggest, however, that once fear symptomatology has been acquired, it manifests in greater elaborated anticipation of predictable threat in the seconds prior to its delivery, as indicated by the association between the SPN to

predictable threat and Time 1 Fear (Michalowski et al., 2015). In sum, different neurobiological markers may be best suited to tracking cross-sectional versus prospective risk for fear and anxiety. In the context of the NPU task, ERPs and startle appear to work well-together to provide insight into multiple processes that may uniquely portend risk for or track current fear versus anxiety. Nonetheless, more work is needed to increase confidence in the specificity of these findings.

4.7. Within-Subjects & Individual Differences

The psychophysiological measures that differentiated predictable and unpredictable threat processing at the within-subjects level differed from the measures that tracked individual/between-subjects variability in prospective symptoms. That is, within-subjects/task effects revealed heightened SPNs to *predictable* versus unpredictable threat cues, and larger startle amplitudes to *unpredictable* versus predictable threat. On the other hand, larger SPNs to *unpredictable* threat were prospectively associated with anxiety, whereas startle during *predictable* threat was prospectively associated with fear. All else being equal, the measures that show the least variation between subjects will yield the most robust within-subjects/task effects (Hajcak et al., 2017). Greater variation between individuals will, on the other hand, reduce the strength of within-subjects effects but will be more suitable to tracking individual differences/correlation with other measures. The results observed here can be understood from this perspective: i.e., measures that are the most sensitive to task effects will not necessarily be those that best distinguish *between* individuals.

4.8. Clinical Implications

Our results provide initial support for predictable and unpredictable threat reactivity as potential liabilities for the development of fear versus anxiety psychopathology, respectively. Specifically, elevated defensive responding to certain threat as well as greater anticipation of uncertain threat may indicate risk for the development and/or worsening of distinct dimensions of psychopathology. Therefore, one implication is that the underlying mechanisms leading to the development and/or worsening of fear and anxiety are different. As such, future interventions could work to target each of these underlying mechanisms individually. Nevertheless, if these findings are found to be trait markers for an inherent vulnerability toward the development of fear versus anxiety, as opposed to indicators of latent psychopathology that has already developed, then these psychophysiological measures could serve as risk markers instead of targets for treatment. Regardless, this work raises questions about the trajectory of the development of these symptoms and provides more information about the underlying structure of fear and anxiety psychopathology.

4.9. Limitations

Despite our findings, our study did have some limitations that future work may wish to address. Firstly, while our focal fear sample allowed us to control for the presence/absence of a focal fear disorder, not all individuals with anxiety disorders meet criteria for focal fear disorders, thus excluding a subsection of individuals with anxiety disorders. Due to this, our findings have limited generalizability to individuals with anxiety but without a focal fear disorder and for patients with a broader range of comorbidities (e.g., externalizing disorders) due to our exclusionary criteria. Secondly,

our Time 2 data was collected during the COVID-19 pandemic which restricted us to online questionnaires not allowing for prospective diagnoses to be made. So, while our prospective results suggest that great reactivity to predictable and unpredictable threat cues may prospectively predict the onset of disorders characterized by fear and anxiety symptomatology, respectively, future work is needed to confirm this hypothesis. Lastly, a longitudinal study design might better assess how changes in neural responses to predictable and unpredictable threat cues covary with increased or decreased symptomatology over time and would provide more detailed information on the causality/directionality of these associations.

5. CONCLUSIONS AND FUTURE WORK[6]

Our findings provide initial support for distinct associations between response to predictable and unpredictable threat and transdiagnostic fear and anxiety. Continued investigation mapping neurobiological response to transdiagnostic fear and anxiety – constructs that may be more homogeneous and more closely tied to mechanism than the categorical disorders – may eventually lead to improved classification and treatment of the internalizing disorders. For example, targeting interventions at unpredictable or predictable threat responding might prove more effective than interventions that focus more generally on overall threat reactivity. Along these lines, prior work has shown that two weeks of selective serotonin reuptake inhibitors (SSRIs) modulates startle response to unpredictable (but not predictable) threat cues in healthy adults (Grillon, Chavis, et al., 2009), suggesting one reason why SSRIs might be more beneficial for individuals with sustained anxiety versus phasic fear. Given that most patients will manifest with symptoms of both fear and anxiety, accurate mapping of the relative contribution of abnormalities in predictable versus unpredictable threat responding to these dimensions may facilitate prescription of personalized treatment protocols. Moreover, greater specification of both treatment targets and their intended effects (i.e., more homogeneous dimensions of psychopathology) should also help ensure that viable treatments are not discarded because they are targeted at more general

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operationalizations of threat reactivity and/or effects are measured in terms of heterogeneous diagnostic categories.

Taken together, our results provide support at multiple neurobiological levels for the theoretical distinction between fear and anxiety symptomatology, and link these dimensions to exaggerated predictable and unpredictable threat reactivity, respectively. Our results support predictable and unpredictable threat reactivity for consideration as prognostic indicators of fear and anxiety symptomatology that may lead to more targeted clinical care.

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APPENDIX

A.1. Diagnostic Analyses

A.1.1. Methods

To facilitate comparison with prior work, we examined associations between Time 1 categorical diagnoses (Focal Fear [specific phobia or performance-only SAD], GAD, SAD) and each condition (no-threat, predictable threat, unpredictable threat), separately for each ERP component and startle. We focused on these diagnoses because they were the most prevalent diagnoses in our sample (Focal Fear, n = 36, SAD, n = 19; GAD, n = 11). In separate logistic regressions, ERPs and startle during no-threat, predictable threat, and unpredictable threat cues (and ISIs for startle) were entered as predictors of presence/absence of diagnosis (dummy coded), controlling for the other diagnoses. All analyses were cross-sectional (Time 1 only), as diagnoses were not obtained on participants at Time 2.

A.1.2. Results

Electrocortical activity

Early SPN. No significant associations were found between the early SPN to no-threat, predictable threat, or unpredictable threat cues and Time 1 diagnosis, ps > .29.

Late SPN. The late SPN to predictable threat cues was associated with a diagnosis of SAD, β = -.149, p =.04, such that individuals with SAD had a larger late SPNs to predictable threat cues, controlling for diagnoses of Focal Fear and GAD, as well as the late SPN to no-threat and unpredictable threat cues. There were no other

significant associations between diagnoses and the late SPN, *p*s > .11.

Startle

No significant associations were found between startle probes delivered during no-threat, predictable threat, or unpredictable threat cues/ISIs and Time 1 diagnoses, *p*s > .13.