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Intolerance of uncertainty and startle potentiation in relation to different threat reinforcement rates

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ABSTRACT

Fear conditioning research on threat predictability has primarily examined the impact of *temporal* (i.e., timing) predictability on the startle reflex. However, there are other key features of threat that can vary in predictability. For example, the *reinforcement rate* (i.e., frequency) of threat is a crucial factor underlying fear learning. The present study examined the impact of threat reinforcement rate on the startle reflex and self-reported anxiety during a fear conditioning paradigm. Forty-five participants completed a fear learning task in which the conditioned stimulus was reinforced with an electric shock to the forearm on 50% of trials in one block and 75% of trials in a second block, in counter-balanced order. The present study also examined whether intolerance of uncertainty (IU), the tendency to perceive or experience uncertainty as stressful or unpleasant, was associated with the startle reflex during conditions of low (50%) vs. high (75%) reinforcement. Results indicated that, across all participants, startle was greater during the 75% relative to the 50% reinforcement condition. IU was positively correlated with startle potentiation (i.e., increased startle response to the CS+ relative to the CS−) during the 50%, but not the 75%, reinforcement condition. Thus, despite receiving fewer electric shocks during the 50% reinforcement condition, individuals with high IU uniquely demonstrated greater defense system activation when impending threat was more uncertain. The association between IU and startle was independent of state anxiety. The present study adds to a growing literature on threat predictability and aversive responding, and suggests IU is associated with abnormal responding in the context of uncertain threat.

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1. Introduction

Fear conditioning is a form of associative learning that is critical to the development and maintenance of anxiety disorders (Lissek et al., 2005). Laboratory studies of fear conditioning often examine differential conditioning, during which multiple conditioned stimuli (CS) are presented, and one is paired with an aversive unconditioned stimulus (UCS) and signals danger (CS+) and the others are not paired and signal safety (CS−). In humans, fear conditioning is often examined using the startle eye blink reflex, which is a cross-species index of defense system activation (Lang et al., 1990). The startle reflex is modulated by emotional valence, such that it is potentiated (i.e., increased) by aversive emotional states and attenuated (i.e., decreased) by appetitive emotional states (Giargiari et al., 2005; Lang et al., 1998). Consistent with these data, fear conditioning studies reliably show that the startle reflex is increased in the presence of the CS+ relative to the CS− (Grillon et al., 1993).

There are several features of threat that can impact defense system activation. The *predictability* of threat has been suggested to delineate the emotional response states of fear and anxiety (Barlow, 2000; Davis, 1992; Grillon et al., 2004). Fear is associated with predictable threat and elicits a fight, flight, or freeze response, whereas anxiety is associated with unpredictable threat and elicits defensive preparedness and hypervigilance. This differentiation has been supported by neuroanatomical (Davis, 1998), neuroimaging (Walker and Davis, 2008), pharmacological (Grillon et al., 2006), and psychophysiological studies (Grillon et al., 2004). The distinction between fear and anxiety also plays an important role in several theoretical perspectives of anxiety disorders. For example, an enhanced fear response in the presence of certain objects or situations is characteristic of phobic disorders, whereas chronic anxious apprehension about the future is typical of generalized anxiety disorder (GAD; Lang et al., 2000).

The majority of research investigating threat predictability has examined the impact of *temporal* (i.e., timing) predictability on the startle reflex (e.g., Grillon et al., 2004). In these paradigms, the exact timing of aversive stimulus delivery is either known (predictable timing condition) or unknown (unpredictable timing condition). However, there are other key features of threat that can vary in predictability. For example, Shankman et al. (2011) found that unpredictable, relative to predictable, shock *intensity* potentiated the startle reflex. To date, most studies have compared predictable and unpredictable threat by using conditions that are matched on the frequency with which an

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aversive stimulus is delivered, but differ in some other dimension (e.g., timing, intensity; Grillon et al., 2004, 2008).

Another experimental feature that can alter the predictability of an aversive stimulus is the *reinforcement rate* (i.e., frequency). Theoretical models of fear conditioning have implicated the reinforcement rate of threat as a crucial factor underlying fear learning (Gallistel and Gibbon, 2000). Indeed, threat responding is based on the possibility that the CS+ is related to impending presentation of the aversive UCS (Norrholm et al., 2006). Therefore, higher reinforcement rates make the UCS more predictable and consequently the CS+/UCS contingency easier to learn, while partial (or decreased) reinforcement of a CS+ increases the number of trials required for fear acquisition and learning (Gallistel and Gibbon, 2000).

The present study examined the impact of 50% and 75% UCS reinforcement rates on the startle reflex and self-reported anxiety during a fear conditioning paradigm. Specifically, 45 participants completed a CS+/CS− threat-of-shock task that contained two within-subjects conditions in a counterbalanced order: 50% shock reinforcement and 75% shock reinforcement. The startle reflex was measured during the CS+ and CS− in each condition, and self-reported anxiety during these conditions was collected retrospectively at the end of the task. Critically, in the 50% reinforcement condition, the UCS was less frequent and therefore more unpredictable; on the other hand, in the 75% condition the UCS was more frequent and predictable. Consistent with prior theoretical models (Gallistel and Gibbon, 2000), we hypothesized that fear-potentiated startle (FPS; i.e., the difference between the CS+ and CS−) and self-reported anxiety potentiation (i.e., the difference between the CS+ and CS−) would be larger in the 75% relative to the 50% reinforcement condition.

We also examined the relationship between individual differences in particular anxiety phenotypes and threat responding. Specifically, we examined how variations in intolerance of uncertainty (IU) related to FPS and self-reported anxiety in both the 50% and 75% condition. IU reflects the tendency to find ambiguity and uncertainty aversive, stressful, and unpleasant (Dugas et al., 2004). IU has been associated with several anxiety disorders, including GAD (Dugas et al., 2004), obsessive-compulsive disorder (OCD; Tolin et al., 2003), panic disorder (Carleton et al., 2013, 2014), posttraumatic stress disorder (PTSD; Fetzner et al., 2013), and social anxiety disorder (SAD; Boelen and Reijntjes, 2009). This has led some researchers to conceptualize IU as a potential transdiagnostic factor of psychopathology (Boswell et al., 2013).

In the present study, participants completed the Intolerance of Uncertainty Scale (IUS; Freeston et al., 1994), and we examined whether IU was associated with FPS and self-reported anxiety in the context of less (50%) versus more (75%) frequent and predictable reinforcement. Since the UCS was less predictable in the 50% condition, we hypothesized that IU would be associated with a heightened startle reflex and self-reported anxiety during the 50% condition. Finally, participants also completed the State Trait Anxiety Inventory (STAI; Spielberger et al., 1983), and we examined whether the association between IU and the startle reflex and self-reported anxiety were *independent* of general symptoms of anxiety. We hypothesized that the relationship between IU and threat responding would remain significant after controlling for anxiety.

2. Methods

2.1. Participants

Forty-five introduction to psychology students participated for course credit. The sample included 32 females (71.1%) and the racial/ethnic distribution was 33.3% Caucasian, 33.3% Asian, 11.1% African American, and 22.2% 'Other'. Informed consent was obtained prior to participation and the research protocol was approved by the Stony Brook University Institutional Review Board.

2.2. Measures

2.2.1. The intolerance of uncertainty scale

The IUS (Freeston et al., 1994) is a 27-item self-report questionnaire that assesses the degree to which individuals find ambiguous or uncertain situations to be stressful and unpleasant. Items are rated on a five-point Likert scale ranging from 1 (*not at all characteristic of me*) to 5 (*entirely characteristic of me*), with higher scores indicating greater IU.

2.2.2. The state trait anxiety inventory

The STAI (Spielberger et al., 1983) is a 40-item self-report measure of anxiety and consists of two 20-item versions measuring state and trait anxiety. Items are rated on a four-point Likert scale ranging from 1 (*not at all*) to 4 (*very much so*), with higher scores indicating greater anxiety. In the present study, participants only completed the STAI-State.

2.3. Stimuli

The startle probe was a 50-ms, 105-dB burst of white noise with instantaneous rise and fall times, delivered binaurally through headphones. Electrical shocks served as the UCS and were delivered to the participant's left forearm. Shocks consisted of 60 Hz constant AC stimulation at an amplitude between 0 and 5 mA presented for 500 ms. In order to ensure that the shocks were significantly aversive to each participant, a workup procedure was used where increasingly stronger shocks were delivered until the participant described it as feeling "highly annoying but not painful." The mean shock level of the final sample was 2.28 mA ($SD = 0.43$).

2.4. Procedure

After obtaining informed consent, participants were seated in front of a 19-in computer monitor inside a sound-attenuated booth and electrodes were attached to measure the startle eye blink reflex. Participants first completed a block of startle habituation trials in which four acoustic probes were delivered in the presence of a fixation cross to elicit initial exaggerated startle responses. After the habituation trials, participants completed a shock workup procedure until a desired shock level was determined.

Next, participants completed two blocks (50% vs. 75% reinforcement) of a fear learning task during which a CS+ (geometric shape) was reinforced with an electric shock to the forearm on 50% or 75% of trials. The CS− (a different geometric shape of the same color) was never paired with an electric shock. The first block of trials included either a green triangle and green star or a blue circle and blue square as conditioned stimuli; the second block of trials included the other set of shapes as stimuli. The pairing between shape and color (e.g. green triangle/star or blue circle/square) were constant; however, CS assignment for each pairing (e.g., whether the green triangle or star was the CS+) was counterbalanced across participants, as was the pair used in each condition (e.g., whether the green triangle/star were the CS in the 50% or 75% conditions). The order of the reinforcement condition (i.e., 50% reinforcement condition first vs. 75% reinforcement condition first) was also counterbalanced across participants. Participants were instructed to passively view the shapes as they appeared, and were told that if they paid attention they could determine which shape was sometimes paired with the shock. Participants were not explicitly informed about the reinforcement schedule (i.e., 50% vs. 75%) in either condition. Each block consisted of 16 trials (8 CS+ and 8 CS−) presented in a random order, and no trial type (CS+ vs. CS−) was presented more than twice in a row. The CS+ and CS− were presented for 6 s; trials were separated by an intertrial interval (ITI) that varied between 2.5 and 3.0 s, during which a fixation cross was presented. During the 50% condition, the CS+ was reinforced with an electric shock on 50% of trials (i.e., 4 of 8 trials). During the 75% condition,

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