Comparing Startle Reactivity in Cannabis Dependent Females with Cannabis Withdrawal Syndrome vs. Non-User Controls Matched on Psychopathology: A Test of the Allostatic Model of Addiction

by

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Abstract

Cannabis Use Disorder (CUD) is expected to increase in the United States, necessitating research examining markers that may predict CUD trajectory. As chronic and harmful cannabis use develops, homeostatic thresholds to stress are altered, resulting in an allostatic load indicative sensitization to acute stress. One physiological marker that may be affected by a greater allostatic load from disordered cannabis use is the startle reflex. Previous studies examining startle reactivity in cannabis users compared to non-users is mixed, possibly due to methodological limitations, such as greater between-subject variability due to a) cannabis use severity, b) differences in internalizing psychopathology, and c) participant sex. The current study aimed to minimize variability between cannabis users and non-users and compare startle reactivity in different threatening contexts. Cannabis users who met for severe CUD with a history of withdrawal were compared to non-using participants matched on psychopathology on startle blink magnitude to no-threat, predictable threat, and unpredictable threat. Results revealed that, compared to non-users, the CUD group demonstrated greater startle blink magnitude to unpredictable and predictable threat during one portion of the startle reactivity task. Current results are consistent with previous alcohol- and nicotine-related studies examining startle reactivity and suggest that greater allostatic load in the CUD group may contribute to sensitization to stressful events. Startle reactivity may be beneficial as a marker to predict CUD trajectory, in addition to being the target for interventions designed to minimize CUD relapse in those seeking abstinence.

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Introduction

Cannabis is quickly becoming one of the most accessible and widely used substances in the United States, in part due to increased nation-wide legalization and decreased public stigma associated with use. Thus, prevalence of Cannabis Use Disorder (CUD) is expected to increase within the next 10-20 years (Carliner et al., 2017; Compton et al., 2016). Greater accessibility of cannabis is thought to indirectly increase risk of CUD, primarily through an increase in cannabis use frequency. For instance, people who use cannabis more frequently, such as daily or weekly use, consistently report greater rates of CUD (Compton et al., 2019), as well as greater CUD rates later in life (van der Pol et al., 2013; 2015), relative to less frequent smokers. Models of addiction posit that people who chronically use substances, such as daily or near-daily users, undergo neurobiological adaptations to circuits responsible for stress responsivity (i.e., allostasis; Koob & Le Moal, 2001), ultimately contributing to substance use disorder chronicity. Unfortunately, examination of psychophysiological stress-related markers of allostasis is sparse among cannabis-using populations. Further, studies examining the allostatic model in cannabis users often differ in their operationalization of disordered cannabis use, possibly contributing to inconsistent findings. Therefore, it is imperative to continue investigating how disordered cannabis use impacts stress-related circuits in individuals who are most likely to experience neurobiological changes indicative of an allostatic load.

Broadly, long-term use of substances leads to a cascade of neurobiological changes that result in worse outcomes when individuals with a substance use disorder attempt to abstain (i.e., greater risk of relapse). Heavily influenced by McEwen's initial conceptualization of allostatic load (McEwen et al., 1998), Koob and Le Moal's (2001) allostatic model of addiction posits that repeated substance use continuously alters neurobiological thresholds responsible for

maintaining homeostasis. As a result of acute substance use, the body quickly attempts to regain homeostasis via a feed-forward process, whereby energy is expended to reset back to equilibrium. Chronic substance use leads to repeated allostatic states, eventually developing into an allostatic load indicative of disordered substance use.

Importantly, allostatic states have unique effects on various stress-related neural systems, depending on the stage of addiction (Koob & Schulkin, 2019). One stage of addiction often studied in substance using samples is the negative affect/withdrawal stage, during which substance deprivation produces greater sensitivity to stress or pain (i.e., hyperkatifeia) (Koob, 2021). Subsequently, motivation for drug use relies on negative, rather than positive, reinforcement to reduce negative affect or symptoms of withdrawal. The transition from positive to negative reinforcement is a result of extensive neurobiological changes to brain regions responsible for threat responding, such as the BNST and central nucleus of the amygdala (Shackman and Fox, 2016). Specifically, the upregulation of stress-related hormone neurotransmission, such as corticotropin-releasing factor (CRF), norepinephrine, and glucocorticoids result in lower thresholds for stress responsivity (for a review, see Koob 2021). Sensitization of these stress circuits is indicative of greater disordered substance use and negative substance-related outcomes, such as risk of relapse during periods of abstinence (Wemm & Sinha, 2019). Taken together, chronic substance use influences neurobiological adaptability by sensitizing the body to acute stress, ultimately increasing the risk of continued substance use via negative reinforcement to reduce stress-related negative affect. As a result of these stress-circuit adaptations, behavioral markers of threat responding become sensitized as well, which are often used as indicators of allostasis.

Startle reactivity is one such marker affected by chronic substance use, and it has been extensively studied among substance using populations. The startle response is an automatic, central nervous system (CNS)-driven response to both cued and uncued aversive stimuli found in mammals. Across species, the startle response likely developed as a way of protecting an organism from potential injury (Grillon, 2008). In humans, the startle response is often measured by ocular blink amplitude to an acoustic startle-probe (i.e., electromyography; EMG; Blumenthal et al., 2005), allowing for a low-cost, efficient way of obtaining a reliable component of the startle reaction under a variety of conditions. Notably, acoustic startle probes during threatening contexts is in-part dependent on trait- and state-like reactions, such that startle reactivity is modulated by individual differences (e.g., substance use disorder severity) and context (e.g., threat predictability).

Regarding context, rodent and human models of startle reactivity often distinguish between anxiety and fear-related aversive states (Grillon, 2008). Broadly, anxiety-related aversive states occur during apprehension of a perceived unpredictable future threat, while fear-related startle occurs within the context of immediate, predictable threat. Three distinct phases have been suggested to drive these anxiety or fear-related responses: potential, distal, and proximal threat (Blanchard et al., 1993; Fanselow, 1986). Both potential and more distal threat (i.e., non-imminent), quantified here as unpredictable threat, elicit sustained anxiety, while imminent, predictable threats elicit a more phasic, fear-oriented response. Differences in threat context appear to activate separate regions of the extended amygdala, which is primarily responsible for threat monitoring and reactivity (Fox et al., 2015; Fox & Shackman, 2019). Specifically, sustained anxiety appears to activate regions such as the bed nucleus of the stria terminalis (BNST), while phasic fear activates the central nucleus of the amygdala. In a sample

of 18 healthy adults, Alvarez and colleagues (2011) found greater dorsal amygdala activation to both unpredictable and predictable threat. However, activation of the BNST occurred only during the unpredictable threat condition, suggesting overlapping, yet distinct, regional neural activation to sustained anxiety (i.e., unpredictable threat) relative to phasic fear (i.e., predictable threat) in humans. In both rodents and humans, unpredictable threat consistently evokes greater startle reactivity than predictable threat (Davis et al., 2010; Grillon, 2008, Grillon et al., 2004), suggesting unique correlates of anxious apprehensive states compared to more acute, fear-oriented states, possibly driven by different brain regions that activate depending on threat context.

In addition to threat predictability, individual differences in substance use severity also impact the startle response. While the allostatic model of addiction suggests chronic substance use leads to neurobiological adaptations to threat responsivity, it also posits that these adaptations continually worsen as addiction severity increases. As such, two people who are diagnosed with a substance use disorder may vary greatly in the extent to which they have undergone such changes, depending on factors such as substance use frequency and chronicity of use. Importantly, the allostatic model of addiction (Koob & LeMoal, 2001) suggests that experiencing withdrawal symptoms during periods of abstinence is an indicator of substance use-related neurobiological adaptations (i.e., downregulation of non-drug reward reactivity and upregulation of stress reactivity). Although there is a substantial literature base examining how substance use affects startle reactivity, discrepant findings between substance using samples, primarily in cannabis users, may result from individual differences in allostatic states.

Startle Reactivity Modulation in Chronic Substance Users

Alcohol and Nicotine

In a series of seminal studies examining the relation between problematic alcohol use and startle reactivity to a threat-of-shock task, Gorka and colleagues' (2013, 2016, 2020) consistently observed potentiated startle reactivity to unpredictable threat, relative to predictable threat. For instance, in two independent samples of adults with a diverse history of psychiatric disorders, Gorka et al. (2016) found that number of past-month binge episodes (zero, one, and two or more) was positively associated with greater startle reactivity to unpredictable threat, but not predictable threat or no threat conditions (study 1). In study 2, they also found that problematic alcohol use, quantified by total scores on the Alcohol Use Disorder Identification Test (AUDIT; Saunders et al., 1993), was positively associated with greater startle reactivity to both unpredictable and predictable threat, though results were trending in a similar direction as study 1. Recently, Gorka and colleagues (2020), replicated and extended this line of research by identifying the brain regions involved in heightened reactivity to unpredictable threat in a sample of 38 individuals with a history of alcohol use disorder (AUD) and 27 individuals without AUD history. Results replicated previous studies by showing greater startle reactivity for unpredictable, relative to predictable or no threat conditions in the AUD sample. Further, startle magnitude during threatening conditions (i.e., both unpredictable and predictable threat) was positively associated with right insula and dorsal anterior cingulate cortex (dACC) Blood-oxygen-level-dependent (BOLD) activation. Notably, the insula and dACC are thought to be part of the anticipatory anxiety network (AAN), a frontolimbic circuit thought to be responsible for responding to uncertain environments (Grupe & Nitschke, 2013).

In addition to alcohol, studies on nicotine deprivation also provide support for potentiated startle reactivity to unpredictable threat (Hogle & Curtin, 2006; Grillon et al., 2007). For

instance, in sample of 38 current nicotine users (19 non-abstinent smokers and 19 overnight-abstinent smokers) and 18 non-smokers, Grillon and colleagues (2007) found potentiated startle reactivity to auditory probes during exposure to unpredictable air blasts delivered to the larynx in abstinent and non-abstinent smokers relative to non-smokers. Interestingly, startle reactivity in abstinent and non-abstinent smokers did not differ, possibly due to a lack of sufficient threat exposure. Importantly, the allostatic model conceptualizes drug deprivation as a state in which stress-related adaptations will be most pronounced, however sufficient threat exposure (i.e., threat of electrical shock vs. threat of air blast) may be necessary to elicit differences between deprived and non-deprived users (Davis et al., 2010). When threat of shock is used during unpredictable threat exposure, deprived smokers demonstrate the expected potentiation of startle reactivity relative to non-deprived smokers (Hogle et al., 2010). Null results between deprived and non-deprived smokers may suggest that unpredictable startle potentiation is not a consequence of chronic substance use, but rather a pre-existing vulnerability factor. Considering current smokers were asked to smoke 30 minutes prior to completing the NPU task, an alternative possibility is that allostasis may exist even during recent substance use. Taken together, results from alcohol and nicotine-using samples demonstrate potentiated startle reactivity to unpredictable threat compared to non-using controls. Further, in alcohol users this exaggerated response is associated with harmful alcohol use outcomes (e.g., number of binge episodes and increased alcohol-related problems).

Cannabis

In dependent cannabis users, changes in startle reactivity as disordered use develops may reflect adaptations to the endocannabinoid system (ECS), which is known to influence stress sensitivity. Specifically, the amygdala is dense with type-1 endocannabinoid receptors (CB1)

(Glass et al., 1997), which function to inhibit the body's natural response to stress (Hillard, 2014). However, chronic cannabis use results in functional tolerance of CB1 receptors, temporarily downregulating CB1 receptor availability as a result of repeated activation by tetrahydrocannabinol (THC), the primary psychoactive compound found in marijuana (Hirvonen et al., 2012; Schlienz et al., 2017). As a result, less CB1 receptor availability in the amygdala contributes to stress sensitization (for a review, see Morena et al., 2016). Moreover, studies of chronic cannabis users consistently find that less CB1 receptor availability in the amygdala is associated with cannabis withdrawal symptoms during early periods of abstinence (D'Souza et al., 2016; Spindle et al., 2021). These findings are consistent with the allostatic load model, which suggests that as disordered substance use becomes more severe, periods of abstinence are associated with greater withdrawal symptoms (Koob & Schulkin, 2019) and risk for relapse (Levin et al., 2010). In other words, withdrawal symptoms in chronic cannabis users may be a marker of allostatic load with respect to changes in stress response circuitry, which could be probed with the startle response under unpredictable vs. predictable threat conditions.

Unfortunately, literature on startle reactivity in cannabis using populations is sparse and overall inconsistent. To the author's knowledge, two studies published on the same sample comparing startle reactivity to contextual threat in regular cannabis users and non-users provide disparate findings. First, Hefner et al. (2018) found greater startle potentiation to unpredictable relative to no threat in the heavy cannabis users compared to the non-using control group, consistent with prior research in samples of problematic alcohol and nicotine users (Gorka et al., 2013; 2020; Grillon et al., 2007). Interestingly, startle potentiation tracked with cannabis use disorder severity and self-reported stress coping motives for cannabis use. However, when attempting to extend these findings in a separate task utilizing unpredictable *and* predictable

threat conditions between heavy users and non-users, Fronk et al (2022) failed to find a group difference in startle potentiation. Presumably, Fronk and Heffner were attempting to recruit cannabis users with likely neurobiological changes as a result of allostatic load, which would predict greater stress sensitivity and startle potentiation to unpredictable threat (Koob & Le Moal, 2001). However, inclusion criteria for the regular cannabis users required smoking cannabis at least five times per week for at least the past six months (Fronk et al., 2022; Heffner et al., 2018). It is possible that there was too much variability between participants in their progression to an allostatic load, which may account for null findings in Fronk et al. (2022). Alternatively, a study that minimizes type two error by requiring all participants to have at least severe CUD, as well as a history of cannabis withdrawal, may decrease this between-participant variability, potentially allowing for more consistent findings in line with alcohol and nicotine studies.

A second limitation of the Fronk et al. (2022) and Hefner et al. (2018) studies is that the sample was comprised of both men and women. Notably, there are considerable sex differences between men and women which influence the trajectory of CUD and experience of cannabis-related withdrawal. For instance, compared to men, women appear to progress more quickly from recreational to harmful cannabis use (i.e., “telescoping effect”) (Hernandez-Avila et al., 2004) and report greater comorbid mood and anxiety disorders (Khan et al., 2013). Further, women appear to be more susceptible to cannabis-related withdrawal (Herrmann et al., 2015; Schleinz et al., 2017), possibly due to hormone-related sex differences and their impact on the endocannabinoid system (Craft et al., 2013; Harte-Hargrove & Dow-Edwards, 2012). Further, risk factors associated with disordered cannabis use and greater withdrawal during abstinence are notoriously understudied in women relative to men (Bonnet & Preuss, 2017). Taken together,

chronic cannabis use is related to neurobiological changes in circuits (e.g., amygdala) responsible for threat responding. Unique sex differences in the ECS and cannabis withdrawal severity have also been found, suggesting studies on threat responsivity in regular cannabis users may benefit from sex-specific samples.

Startle Reactivity and Internalizing Psychopathology

It is also possible that failure to take into account comorbid psychopathology may be responsible for mixed startle reactivity findings in cannabis users. Specifically, differences in startle reactivity between sustained (i.e., unpredictable threat) and phasic (i.e., predictable threat) fear in rodents parallel a two-factor model of internalizing disorders that include anxious-misery and fear-based symptomology (Davis et al., 2010). Anxious-misery disorders include symptoms indicative of anxious apprehension, often observed as persistent and intrusive ruminative thoughts or worries. Fear-related disorders, on the other hand, suggest the perception of more imminent danger and greater temporary parasympathetic activation. Evidence from confirmatory factor analyses (Krueger, 1999; Vollebergh et al., 2001; Zinbarg & Barlow; 1996) and genetic-based studies (Kendler et al., 1992; Scherrer et al., 2000), suggest similar latent dimensions and genetic compositions within anxious-misery psychopathology (i.e., major depressive episode, dysthymia, and generalized anxiety disorder) as well as fear-related disorders (i.e., social and specific phobias, panic disorder). Further, both anxious-misery and fear-related disorders appear to converge onto one "internalizing" factor, often associated with general negative affectivity (Krueger, 1999; Watson & Clark, 1984). Though likely part of one internalizing factor, anxious-misery and fear-related disorders appear to differentially influence startle reactivity to unpredictable and predictable threat.

Broadly, psychopathology indicative of chronic generalized distress is reflective of a more blunted startle response, while fear-based, phobic disorders are more reflective of a potentiated response (Cuthbert et al., 2003; Lang & McTeague, 2009; McTeague et al., 2009; McTeague & Lang, 2012). To help explain this discrepancy, models of internalizing psychopathology suggested one overarching dimension of negative affectivity with anxious-misery and fear-related disorders at each end of the spectrum (Lang & McTeague, 2009). Indeed, work from Lang and colleagues often examined startle responsivity via acoustic probes during fearful or threatening imagery and consistently found evidence supporting a dimensional model of internalizing psychopathology that was able to differentially predict startle reactivity between disorders. For instance, in a sample of 119 participants diagnosed with either an anxious disorder (i.e., panic disorder with agoraphobia and GAD) or fearful disorder (i.e., specific or social phobia), those with an anxious disorder demonstrated blunted startle reactivity relative to those with a fearful disorder (Lang & McTeague, 2009). In an extension of these studies, McTeague and colleagues (2009) compared startle reactivity to imagined threat between people diagnosed with social phobia with and without depression. Consistent with a dimensional model of internalizing psychopathology, results revealed that depression attenuated startle reactivity relative to those with social phobia without depression.

In addition to anxious-misery and fear-related disorders, trauma-exposure appears to influence startle reactivity in those with posttraumatic stress disorder (PTSD), further suggesting the differential role of acute vs. sustained stress experience on the modulation of startle reactivity. McTeague and colleagues (2010) compared participants without a trauma history and those exposed to a traumatic event without PTSD, and those with PTSD reporting either one index trauma (single-trauma participants) or those with a history of multiple traumatic events

(multiple-trauma participants). Authors found blunted startle response to aversive cues in participants with recurrent trauma experiences, while those with just one traumatic event demonstrated a potentiated response. Overall, these data support a dimensional model of negative affectivity by showing that those with generalized anxiety and persistent stress experience (i.e., multi-trauma history) demonstrate blunted startle reactivity to threat, while more acute (i.e., single-trauma history), fear-related psychopathology potentiates this response (McTeague & Lang, 2012).

Though Lang and colleagues' work provides evidence that anxious-misery and fear-based disorders appear to have opposite effects on startle reactivity, their studies primarily utilize an imagined threat paradigm during acoustic probe onset to elicit a startle response. This methodology varies greatly from more recent studies that tend to use an electrical shock as the threatening stimuli, most commonly in the form of the NPU paradigm (Schmitz & Grillon, 2012). Research utilizing the NPU task to examine startle reactivity between anxious-misery and fear-based disorders is mixed. For instance, those with panic disorder have demonstrated greater startle reactivity during both unpredictable and predictable threat conditions in one study (Shankman et al., 2013), but only the unpredictable threat condition in another study (Grillon et al., 2008). Further, Grillon and colleagues (2009) found that patients with PTSD had greater startle reactivity during unpredictable threat relative to those with GAD. However, both groups demonstrated potentiated startle response to predictable threat, which is inconsistent with the studies from Lang and colleagues (2009). Finally, Melzig et al., (2007) found that participants with comorbid panic disorder and depression were more likely to exhibit a blunted startle response during anticipation of unpredictable threat, consistent with previous work finding that depression may be best categorized as part of the anxious-misery disorders (Krueger 1999) and

would be expected to blunt startle reactivity to threat (Lang & McTeague, 2009; McTeague et al., 2009). To summarize, there is ample evidence for a dimensional role of internalizing psychopathology that appears to influence startle reactivity. Importantly, previous work examining startle reactivity in substance using samples seldom takes into account current psychological disorders, which may partially explain inconsistent findings.

Current Study

Startle reactivity has been extensively studied in chronic substance users and those with internalized psychopathology. For substances such as alcohol or nicotine, previous research consistently shows potentiated startle reactivity to unpredictable threat in users compared to non-using controls. However, studies examining cannabis users' startle reactivity are mixed (Fronk et al., 2022; Hefner et al., 2018), possibly due to several reasons. First, participants in these studies were only required to be regular cannabis users (e.g., using at least twice per day, five days per week, over the past six months). Though frequent cannabis use was established, participants could still vary greatly in their allostatic load, particularly if there were individual differences in experience of withdrawal during abstinence. To minimize between subject variability in allostatic load and maximize the chance of finding results consistent with nicotine and alcohol studies on startle reactivity, cannabis users who are most likely to demonstrate an allostatic load should be compared to non-users. In regular cannabis users, this sample would be comprised of 1) females, due to their heightened risk of more severe cannabis withdrawal and to limit sex-related variability in allostatic load, 2) cannabis users with severe CUD due to theorized worsening allostasis in more severe addiction, and 3) users with a history of cannabis withdrawal syndrome during periods of abstinence, which reflects neurobiological adaptations indicative of allostasis.

A second reason for mixed findings could be due to participants' current psychopathology interacting with CUD-related allostasis to influence startle reactivity. For those with internalizing psychopathology, researchers observe an overall blunting of startle reactivity during unpredictable threat conditions in those with anxious-misery disorders, and a potentiated response in those with fear-related disorders. Internalizing psychopathology, which is highly comorbid with CUD, was not taken into account in either the Fronk (2022) or Hefner (2018) studies. Thus, by comparing startle reactivity between severe CUD users with non-users who matched on psychopathology, findings are more likely to be attributable to allostatic changes resulting from chronic substance use.

Taken together, the current study sought to replicate and extend previous work evaluating the allostatic model of addiction. Specifically, we aim to examine group differences in a reliable marker of stress reactivity (i.e., startle response) in CUD participants at elevated risk of demonstrating an allostatic load (i.e., females with severe CUD and a history of withdrawal), vs. a non-cannabis using control group matched on psychopathology.

Methods

Power Analysis

G*Power (Erdfelder et al., 1996) was used to conduct an *a priori* power analyses in order to assess how large the sample should be to test our primary hypothesis that startle-blink amplitude in the unpredictable shock condition will differ between the CUD and control groups. An alpha of .05 and statistical power of .80 were selected. Following previous work examining stress-related increases in startle reactivity to unpredictable threat in alcohol users vs. non-users (Gorka et al., 2016), an effect size of $d = 3.2$ was used for the current power analysis.

Conservatively, we plan to power a repeated-measures analysis of variance (RM-ANOVA) to detect a medium effect size for a group (CUD vs. Control)*Condition(unpredictable vs. predictable vs. no-shock) interaction. G*Power suggested a total sample size of $N = 28$, or a minimum of 14 participants per group.

Participants and Procedure

Participants were recruited from Auburn University and the surrounding Auburn/Opelika community. Students from Auburn University will be recruited via SONA, while flyers were posted in in the Auburn/Opelika area for interested participants from the community. Importantly, the allostatic load model of addiction suggests that frequent and chronic substance use is most likely to lead to neurobiological changes indicative of disordered use (Koob & Schulkin, 2001; 2019). As such, the current study recruited daily cannabis users with severe CUD (i.e., six or more symptoms of CUD). Further, physiological dependence on cannabis is most apparent in those with a history of withdrawal, since withdrawal symptoms indicate downregulation of endogenous cannabinoid receptors (for a review, see Budney et al., 2004), which may result in dysregulated reward and threat responding. Thus, participants in the cannabis-using group were also required to have at least a lifetime history of cannabis-related withdrawal. Second, it is possible that sex differences may influence startle reactivity in severe CUD users, considering prominent sex differences in cannabis use trajectory (Hernandez-Avila et al., 2004) and experience of withdrawal (Herrmann et al., 2015; Schleinz et al., 2017). Therefore, the current study only recruited female participants since they appear more vulnerable to cannabis-related withdrawal. Finally, since fear- and anxious-misery-related disorders differentially influence startle reactivity (Lang & McTeague, 2009), participant groups were matched on psychodiagnostics status. Following similar startle reactivity studies comparing

groups with varying psychodiagnostic status (Gorka et al., 2018), groups in the current study were ratio-matched on current psychological disorder status.

Inclusion criteria for both groups (i.e., severe CUD group and control group) included being between 18-30 years old and fluent in English, while exclusion criteria included: 1) other non-prescribed substance use, with the exceptions of alcohol and nicotine, more than once per month on average over the past year, 2) past year moderate or severe Alcohol Use Disorder (i.e., >=4 criteria), 3) history of psychotic, seizure, or cardiovascular disorder, 4) allergies to lotions/cosmetics, 5) change in psychotropic medication in the past month, 6) current daily use of prescribed anti-psychotic, mood stabilizer, anticonvulsant, or benzodiazepine medication, 7) pregnancy, 8) and current suicidal intent, as measured by the Beck Depression Inventory – 2 (BDI-2) and Columbia-Suicide Severity Rating Scale (C-SSRS). Exclusion criteria for the CUD group are as follows: Daily cannabis use, on average, over the past year, cannabis use on at least 24 out of the past 28 days, lifetime history of cannabis withdrawal, use of cannabidiol (CBD) at least 10 or more times in the past month, immediate plan to discontinue cannabis use, and those currently receiving treatment for cannabis use-related problems.

Interested participants completed an online screener to assess potential study eligibility. Those found to be eligible from the online screener were invited to participate in a 2-hour long in-person interview to assess past year psychopathology using the Mini International Neuropsychiatric Interview (MINI; Sheehan, 1998), in addition to CUD criteria for the severe CUD group. Eligible participants then scheduled a baseline EEG appointment, where they will complete a battery of measures relevant for the larger study, as well as computerized tasks while EEG and EMG data were collected.

Measures

Timeline Follow-Back

To assess past-month substance use, participants completed the Timeline Follow-Back (TLFB; Sobell & Sobell, 1996). Specifically, participants reported how many cannabis sessions they engaged in per day over the past 30 days, in addition to how many days they used alcohol and nicotine. The TLFB appears to reliably measure self-reported cannabis and nicotine use frequency (Robinson et al., 2014), as well as alcohol frequency (Sobell & Sobell, 1996).

Semi-Structured Interviews

Current psychopathology was assessed via the Mini International Neuropsychiatric Interview (MINI; Sheehan, 1998), while past-year CUD criteria for the severe CUD group was assessed by the Structured Clinical Interview for the DSM-5, Research Version (SCID-5-RV; First et al., 2015). All interviews were administered by trained graduate-level clinicians. Training to administer the SCID-5-RV included practice sessions, demonstration videos, and weekly reviews of all CUD diagnoses with a licensed clinical psychologist. All criteria were coded as either 0 (not present) or 1 (present).

No Shock, Predictable Shock, and Unpredictable Shock Task (NPU Task)

The NPU task is a validated 20-minute task designed to assess startle blink amplitude as an index of threat reactivity (Kaye et al., 2016; Schmitz & Grillon, 2012). The task is separated into three conditions (no-shock, predictable shock, unpredictable shock) that differ in shock occurrence and predictability (see Figure 1). In the no shock condition, no shocks are delivered. In the predictable shock condition, shocks are reliably administered only during a particular visual cue. In the unpredictable shock condition, shocks are unpredictably administered at any

point during the condition. Each of the three conditions lasts approximately 150 seconds each and each condition is presented twice. Auditory startle probes are presented in each of the conditions in order to quantify startle blink amplitude in differing threat contexts (i.e., unpredictable shock threat vs. predictable shock threat vs. no shock threat. In total, participants received 60 auditory startle probes (20 per condition) and 12 shocks (6 per threat-related condition).

Prior to beginning the NPU task, participants completed a shock work-up procedure to ensure the shock they experience is considered aversive. To this end, participants rated incrementally increasing shocks on a scale from 0 to 100, where 0 is that they did not feel anything at all, while 100 is considered the maximum shock intensity that they consider to be highly annoying but not painful. Considering startle reactivity may be attenuated after several acoustic startle probes (i.e., habituation; Davis and File, 1984), participants were then presented with a habituation procedure, in which nine startle probes will be presented within a 150 second period.

Data Pre-Processing

EMG blink amplitude data processing will follow Blumenthal et al.'s (2005) guidelines, including the application of a 28-Hz high-pass filter, rectification, application of a 40-Hz low-pass filter to smooth data, segmentation from -50 to 150 ms, and baseline correction (i.e., subtraction of the average activity at 50 ms prior to probe onset). After onset of the auditory probe, a simple MinMax marker algorithm identified peak amplitude between 20 to 150 ms. As an additional precaution to preserve accuracy, peak amplitudes were manually checked. To ensure each peak was a product of blink reaction to the startle probe, blinks were considered

missing for the following: peak amplitudes |20| uV before 10ms or if an identified peak occurred before 20 ms. Nonresponses, in which peaks were coded as 0, included peaks that were less than 10 uV. Analyses included all acceptable blink magnitude values (i.e., all acceptable averaged values, including non-responses).

Data Analytic Plan

First, group differences were examined to ensure participant groups are equally matched on psychiatric diagnoses, in addition to other variables of interest (e.g., age, past-month alcohol/nicotine use). Next, a two-way (3x2) mixed repeated measures Analysis of Variance (RM-ANOVA) was conducted as a manipulation check to ensure startle reactivity across threat conditions (No Shock, Predictable Shock, Unpredictable Shock) and Cue Type (CD, ISI) differed. To test for an effect of group, a three-way (2x3x2) mixed RM-ANOVA was used, with one between-subjects factor (CUD vs. Control groups) and two within-subject factors (Condition [No Shock, Predictable, Unpredictable] and CueType [ISI, CD]). For all RM-ANOVAs, Mauchly's test of sphericity was examined to ensure that homogeneity-of-variance-of-differences assumption was not violated. If this assumption was violated, then Greenhouse-Geisser correction was applied.

In the case of a significant Group*Condition interaction, specificity of effects to the unpredictable shock condition was examined with two post hoc Fisher least significant difference tests (i.e., unpredictable threat vs. no-shock and unpredictable threat vs. predictable threat). Considering there is the threat of shock during both ISI and CD in the Unpredictable shock condition, startle response magnitude was averaged between ISI and CD when comparing Unpredictable to the No-Shock condition. Therefore, the first post-hoc test compared

Unpredictable (averaged across ISI and CD) vs. No Shock (averaged across ISI and CD) between CUD and Control groups. Since threat is only present at CD in the Predictable shock condition, the second post-hoc test compared Unpredictable CD vs. Predictable CD between the CUD and control group. Considering individual differences in baseline startle reactivity may influence startle magnitude to both predictable and unpredictable threat (Bradford et al., 2014), baseline startle reactivity was added as a covariate for all analyses. Finally, exploratory analyses were also conducted to test the robustness of any significant findings by examining the exclusion of participants considered "non-responders," examining raw startle amplitude value (i.e., all acceptable averaged values, excluding non-responses), and utilizing a T-score transformation to create within-subject standardized condition values to account for any outlier blink responses (Kaye et al., 2016).

H_0: Group differences will not emerge between the CUD and matched control groups for all three conditions of the NPU task.

H_1: Group differences will emerge between the CUD and matched control group. Specific group differences will show the CUD group demonstrates *amplified* EMG-measured startle response that is specific to the unpredictable shock condition, relative to the control group.

Results

Demographics and Descriptives

Skewness and kurtosis for blink magnitude in the No-Shock condition and ISI cue type was marginally positively skewed (skewness=2.02), while all other condition and cue type values were <2. All blink magnitude values appeared leptokurtic (all kurtosis values >2). Examination of boxplots utilizing Tukey's hinges revealed one participant that was considered an outlier for

every condition and cue type pair. This participant was removed from all analyses (final sample *N*=40). Re-evaluation of skewness and kurtosis revealed only the No-Shock Countdown blink magnitude value was slightly leptokurtic (kurtosis=2.03), while all other values were normally distributed (skewness <1.32, kurtosis <2). Demographics and relevant clinical descriptives can be found in Table 1. Compared to the control group (ICAE-C), the current cannabis using group (ICAE) were older, reported greater past-month alcohol use, and greater past-month nicotine use. Groups were matched on current psychiatric diagnoses. Fisher's exact significance tests revealed no group differences in the proportion of diagnostic status for all psychological disorders between groups (all ps > .41). To examine whether age, past-month alcohol use, and past month nicotine use were significantly associated with blink magnitude values, three separate RM-ANOVAs were conducted to examine whether each of these variables demonstrated a main effect of condition or cue type, or their interaction. Results revealed that there was not a significant main effect for age, past-month alcohol use, and past-month nicotine use for condition, cue type, or their interaction (all ps >.06). Therefore, these variables were not added as covariates in primary analyses.

Effect of Task Condition and Cue Type on Blink Magnitude Manipulation Check

Blink magnitude means and standard deviations between groups for each condition (No-Shock, Predictable Shock, Unpredictable Shock) and cue type (CD, ISI) can be found in Table 1. Mauchly's test of sphericity indicated there was not a violation of the homogeneity-of-variance-of-differences assumption for condition, $\chi^2(2)=4.49$, p=.11 or the condition*cue type interaction, $\chi^2(2)=5.12$, p=.08, therefore degrees of freedom were not corrected. Across the cannabis-using and non-using groups, there was a significant main effect of condition after covarying for habituation magnitude ($F[2, 79] = 5.24$, $p = .01$, $\eta_p^2 = .12$). However, the main effect of cue type

($F[1, 39] = 2.16$, $p = .15$, $\eta_p^2 = .05$) and a condition*cue type interaction ($F[2, 79] = 0.41$, $p = .66$, $\eta_p^2 = .01$) were insignificant.

Across groups and cue types, blink magnitude during the unpredictable shock condition was significantly greater than the predictable shock condition (mean U-P difference=28.98, p<.001) and the no shock condition (mean U-N difference=40.89, p<.001). Further, blink magnitude during the predictable shock condition was significantly greater than the no shock condition (mean P-N difference= 11.91, p=.005; U>P>N). Overall, despite insignificant cue type or condition*cue type interaction effects, the NPU task manipulation successfully influenced blink magnitude within each condition and cue type, such that blink magnitude values were greater during the unpredictable shock condition relative to the predictable and no-shock conditions for both CD and ISI cue types.

Effect of Task Condition and Cue Type on Blink Magnitude Between Groups

Mauchly's test of sphericity indicated there was not a violation of the homogeneity-of-variance-of-differences assumption for condition, $\chi^2(2)=4.16$, p=.13, or the condition*cuetype interaction, $\chi^2(2)=3.56$, p=.17, therefore degrees of freedom were not corrected. The main group between subjects effect was not significant ($F[1,39]=0.11$, p=.74). In contrast to hypotheses, the condition*group interaction ($F[1, 39] = 1.19$, $p = .31$, $\eta_p^2 = .03$) was not significant; however, the cue type*group interaction ($F[1, 39] = 4.89$, $p = .03$, $\eta_p^2 = .12$) and the condition*cue type*group interaction ($F[2, 79] = 3.704$, $p = .03$, $\eta_p^2 = .09$) were significant.

Next, unpredictable (i.e., unpredictable – no shock) and predictable (predictable – no shock) threat potentiation values were examined separately by cue type and Group to probe the Condition*Cue Type*Group interaction. Mean difference values can be found in Figure 3.

Within the CD cue type, the CUD group displayed numerically greater threat-related blink potentiation magnitudes during the unpredictable shock condition (mean difference=57.21, SE=9.57, p<.001) relative to the Control group (mean difference=31.49, SE=9.57, p=.002), consistent with hypotheses. However, the Group*Condition interaction was trending, but non-significant ($F[1, 39] = 3.58$, $p = .07$, $\eta_p^2 = .09$). Similarly, the CUD group also displayed numerically greater threat-related blink potentiation during the predictable shock condition (mean difference=32.32, SE=8.72, p<.001) relative to the Control group (mean difference=9.87, SE=8.72, p=.27), though the Group*Condition interaction was a non-significant trend, ($F[1, 39] = 3.29$, $p = .08$, $\eta2 = .08$). Within the ISI condition, the CUD group displayed comparable threat-related blink potentiation magnitudes during the unpredictable shock condition (mean difference= 39.64, SE=6.67, p<.001) relative to the Control group (mean difference=35.22, SE=6.67, p<.001), ($F[1, 39] = 0.22$ $p = .64$, $\eta_p^2 = .01$). Similarly, the CUD group also displayed comparable threat-related blink potentiation during the predictable shock condition (mean difference=-3.04, SE=6.43, p=.64) relative to the Control group (mean difference=8.51, SE=6.43, p=.19), ($F[1, 39] = 1.60$, $p = .21$, $\eta_p^2 = .04$).

Robustness Analyses

Three exploratory analyses were conducted to examine the robustness of the Group*Condition*CueType interaction: a) removing participants considered “non-responders,” or those that had less than 50% non-zero blink magnitude values across conditions and cue types, b) examining blink amplitude (i.e., values that do not account for non-responses) instead of magnitude values, and c) examining T-score blink magnitude and amplitude values.

Removing Non-Responders

Two participants were determined to have less than 50% blink magnitude non-zero responses across each condition and cue type and were subsequently removed for this analysis (total sample *N*=38, CUD group *n*=19, Control group *n*=19). Mauchly's test of sphericity indicated there was not a violation of the homogeneity-of-variance-of-differences assumption for condition, $\chi^2(2)=3.87$, p=.14, or the condition*cuetype interaction, $\chi^2(2)=3.47$, p=.18, therefore degrees of freedom were not corrected. After covarying for habituation magnitude, the main effect of condition ($F[2, 75] = 4.95$, $p = .01$, $\eta_p^2 = .12$) was significant, while the main effect of cue type ($F[1, 37] = 2.26$, $p = .14$, $\eta_p^2 = .06$) and the condition*cue type interaction ($F[1, 37] = 0.47$, $p = .63$, $\eta_p^2 = .01$) were not significant. Finally, the condition*group interaction ($F[1, 37] = 1.02$, $p = .36$, $\eta_p^2 = .03$) remained non-significant; however, the cue type*group interaction ($F[1, 37] = 4.91$, $p = .03$, $\eta_p^2 = .12$) and the condition*cue type*group interaction ($F[2, 75] = 3.75$, $p = .03$, $\eta_p^2 = .10$) remained significant.

Blink Amplitude Values

Skewness and kurtosis between blink amplitude and blink magnitude values were similar, in addition to revealing the same participant as an outlier after examining blink amplitude boxplots. Therefore, this participant was removed for subsequent analyses . Further, two participants were removed due to having all non-response values in at least one condition/cue type variables of interest (*N*=38). Mauchly's test of sphericity indicated there was not a violation of the homogeneity-of-variance-of-differences assumption for condition, $\chi^2(2)=3.77$, p=.15, or the condition*cuetype interaction, $\chi^2(2)=4.40$, p=.11, therefore degrees of freedom were not corrected. After covarying for habituation amplitude, the main effect of condition ($F[2, 75] = 4.13$, $p = .02$, $\eta_p^2 = .11$) was significant, while the main effect of cue type ($F[1, 37] = 1.18$, $p = .29$, $\eta_p^2 = .03$) and the condition*cue type interaction ($F[1, 37] = 0.69$, $p = .51$, $\eta_p^2 = .02$) were

not significant. Finally, the condition*group interaction (F[1, 37] = 0.94, p = .39, η_p^2 = .03) remained non-significant; however, the cue type*group interaction (F[1, 37] = 5.21, p = .03, η_p^2 = .13) remained significant. Finally, the condition*cue type*group interaction (F[2, 75] = 3.18, p = .048, η_p^2 = .08) remained significant.

T-Score Transformed Blink Magnitude/Amplitude Values

T-score transformed blink magnitude values were examined as a more conservative approach to account for outlier blink values (Gorka et al., 2013). Mauchly's test of sphericity indicated there was not a violation of the homogeneity-of-variance-of-differences assumption for condition, $\chi^2(2)=1.85$, p=.40, or the condition*cuetype interaction, $\chi^2(2)=2.82$, p=.24, therefore degrees of freedom were not corrected. The main effect of condition (F[2, 80] = 63.35, p <.001, $\eta 2$ = .62) and cue type (F[1, 40] = 44.83, p<.001, $\eta 2$ = .54), as well as the condition*cue type interaction (F[2, 80] = 4.55, p=.01, $\eta 2$ = .10) were significant. The condition*group, cuetype*group, and group*condition*cuetype interactions were all non-significant (ps>.12). Despite being non-significant, means differences were examined to assess whether the T-scored blink magnitude values demonstrated a similar pattern to the raw blink magnitude group*condition*cuetype interaction. Consistent with findings from the raw blink values, within the CD cue type the CUD group demonstrated numerically greater unpredictable (mean difference = 9.56, SE=1.32, p<.001) and predictable (mean difference = 4.70, SE=1.12, p<.001) potentiation compared to the Control group unpredictable (mean difference =6.67, SE=1.29, p<.001) and predictable (mean difference =2.47, SE=1.09, p=.03) potentiation. Within the ISI cue type, the CUD group demonstrated comparable unpredictable (mean difference=7.53, SE=1.05, p<.001) and predictable (mean difference=-0.1, SE=1.08, p=.93) potentiation

compared to the Control group unpredictable (mean difference=6.08, SE=1.02, p<.001) and predictable (mean difference=1.54, SE=1.06, p=.15).

Next, T-score transformed blink amplitude values were examined. Mauchly's test of sphericity indicated there was not a violation of the homogeneity-of-variance-of-differences assumption for condition, $\chi^2(2)=1.60$, p=.45; however, the condition*cue type interaction was significant, $\chi^2(2)=7.53$, p=.02, therefore degrees of freedom were corrected by utilizing a Greenhouse-Geisser correction. The main effect of condition ($F[2, 77] = 51.76$, $p < .001$, $\eta 2 = .58$) and cue type ($F[1, 38] = 43.80$, $p < .001$, $\eta 2 = .54$) were significant; however, their interaction was trending but not significant($F[2, 77] = 2.57$, $p = .08$, $\eta 2 = .07$). The condition*group, cuetype*group, and group*condition*cuetype interactions were all non-significant (ps>.08). Similar to T-scored blink magnitude values, mean differences were examined to see if these findings exhibited a similar pattern to the raw blink amplitude values. Within the CD cue type, the CUD group demonstrated numerically greater unpredictable (mean difference=9.04, SE=1.33, p<.001) and predictable (mean difference=4.22, SE=1.18, p=.001) potentiation compared to the Control group unpredictable (mean difference=6.68, SE=1.29, p<.001) and predictable (mean difference=2.39, SE=1.15, p=.046) potentiation. Within the ISI cue type, the CUD group demonstrated comparable unpredictable (mean difference=6.73, SE=1.09, p<.001) and predictable (mean difference=-0.32, SE=1.21, p=.79) potentiation compared to the Control group unpredictable (mean difference=6.18, SE=1.06, p<.001) and predictable (mean difference=2.39, SE=1.18, p=.05) potentiation.

Discussion

The current study is the first to examine differences in startle reactivity between CUD users and non-users while accounting for common variables known to influence allostatic load propensity (i.e., participant sex, severe CUD status, history of withdrawal) and startle reactivity (i.e., psychodiagnostics status). Overall, the primary hypothesis was partially supported. Specifically, the 2x3x2 RM-ANOVA revealed a significant Group*Condition*CueType interaction, suggesting significant group differences in condition's effect (No-Shock, Predictable Shock, Unpredictable Shock) on startle amplitude depending on cue type (CD or ISI). Post-hoc mean difference tests revealed that the CUD group demonstrated larger blink potentiation to unpredictable and predictable threat relative to the Control group, but this effect was only present during the CD cue type. Further, robustness analyses which removed participants considered "non-responders," as well as analyses that examined blink amplitude instead of blink magnitude, produced equivalent results.

To the author's knowledge, only two previous studies have examined startle reactivity to unpredictable threat relative to no threat in cannabis users and non-users. Hefner et al. (2018) found evidence of greater potentiation to unpredictable threat in cannabis users compared to non-users, consistent with previous alcohol (Moberg et al. 2017) and nicotine (Grillon et al., 2007) studies. Unfortunately, Hefner et al. (2018) did not examine group differences in potentiation to predictable threat; however, when examining unpredictable and predictable threat potentiation between heavy cannabis users and non-users, Fronk and colleagues (2022) found no group differences. Current study results appear to replicate and extend Hefner and colleagues' (2018) findings by demonstrating startle potentiation to both unpredictable *and* predictable threat in cannabis users compared to a non-using control group. Notably, Hefner et al. (2018) found that heavy cannabis users demonstrated blink startle potentiation that was 15uV greater during

unpredictable threat than the non-using group. In the current study, the CUD group demonstrated potentiation to unpredictable threat that was 25.72uV greater than the control group during the CD cue type; despite post-hoc tests showing this effect was trending (.07) but not significantly different between groups, results are overall consistent between studies and suggest heavy or disordered cannabis users appear to demonstrate greater stress sensitivity (i.e., startle potentiation) to generalized threat compared to non-users.

Greater startle potentiation to unpredictable and predictable threat in the CUD group provides further support for the allostatic model of addiction, which proposes that chronic substance use repeatedly alters homeostatic thresholds of neurobiological and physiological systems responsible for stress responding, leading to an allostatic load indicative of dysregulated stress reactivity (Koob & Le Moal, 2001). It is possible that chronic cannabis use results in temporary downregulation of CB1 receptors in the amygdala (Morena et al., 2016) or decreased production of the endocannabinoid anandamide (Morgan et al., 2013), ultimately resulting in greater reactivity to phasic and sustained threat. Notably, the current study utilized a well-known measure of the body's stress response (i.e., blink startle magnitude) that differs between individuals depending on a variety of factors, such as psychiatric diagnosis status. Groups were matched on psychopathology; thus, findings are less likely to be attributed to anxious-misery or fear-related disorders impacting startle reactivity but rather reflect group differences in allostatic load affecting stress reactivity.

Unique to the current study, the CUD group demonstrated greater startle potentiation to predictable threat relative to the control group, which contrasts Fronk et al.'s (2022) findings that found no group differences between unpredictable or predictable shock conditions for either a similarly structured countdown cue type or interstimulus interval cue type. However, several

methodological differences between the current study and Fronk and colleagues' study may explain result discrepancies. For instance, Fronk et al.'s (2022) study procedures did not include assessment of current psychopathology, which is known to heavily influence startle reactivity to different threat types. Further, inclusion criteria for heavy cannabis users included smoking at least five days per week and at least two times per day of use for the past year. This may have led to greater variability in allostatic load, which theoretically could affect startle potentiation. Finally, the heavy cannabis using sample (n=66) in Fronk's study was evenly split between men and women. Sex differences in psychopathology and cannabis use severity may further increase variability in startle potentiation, ultimately leading to null findings between groups. Current findings may suggest that allostatic load effects on startle potentiation are subject to individual differences in the cannabis user population.

Previous studies examining startle reactivity in other substance-using samples have primarily found greater startle magnitude to only unpredictable threat (Gorka et al., 2020) and, in some instances, unpredictable threat potentiation is larger relative to predictable threat in alcohol using samples (Moberg et al., 2017). Comparable startle potentiation to both unpredictable and predictable threat in the CUD group relative to non-using controls during the CD cue type may suggest that cannabis users demonstrate general threat-related potentiation, as opposed to specific potentiation to unpredictable threat. Threat type (i.e., unpredictable vs. predictable) is known to differentially activate brain regions responsible for threat appraisal, sustained threat response, and hypervigilance. For instance, Alvarez and colleagues (2011) found that unpredictable and predictable threat temporarily activated the dorsal amygdala, but only unpredictable threat elicited activity in regions responsible for sustained fear responses (bed nucleus of the stria terminalis) and hypervigilance (anterior insula/frontoparietal cortical

network). It is possible that disordered cannabis use is related to alterations in phasic and sustained fear reactivity due to neurobiological changes to the ECS that affect reactivity to both unpredictable and predictable threat; however, future work is needed to fully understand how chronic cannabis use influences the ECS and startle reactivity.

Current findings also revealed group differences in startle potentiation between cue types during the NPU task. Between groups, those in the CUD sample demonstrated greater potentiation for both threat types during the CD cue type compared to the control group, though this group difference was a non-significant trend. In contrast, CUD and Control groups had comparable threat potentiation during ISI. Examining means differences in startle potentiation revealed the significant group*condition*cue type interaction may have been driven by greater potentiation to the CD relative to ISI cue (see figure 3). These results are in contrast to a previous study that found participants with problematic alcohol use and a current diagnosis of panic disorder exhibited greater startle potentiation to unpredictable threat during the ISI (but not CD) cue type, relative to healthy controls (Gorka et al., 2013). Gorka and colleagues (2013) suggest a possible carry-over effect between predictable and unpredictable threat during CD since this cue type always contains a shock during the predictable threat condition and may therefore be a "strong situation" (Cooper and Withey, 2009).

Greater startle potentiation to threat during the countdown period in disordered cannabis users may be indicative of altered fear learning, such that the countdown period is more strongly associated with threat compared to the ISI period in the CUD group. It is possible that alterations in the endocannabinoid system resulting from chronic use influence aspects of fear conditioning. For instance, greater cannabis use has been shown to decrease levels of the endocannabinoid anandamide (Morgan et al., 2013), which is positively correlated with fear extinction (Spohrs et

al., 2021). Further, chronic cannabis users have been found to demonstrate worse fear extinction measured by skin conductance compared to a matched, non-using control group (Papini et al., 2017). In the current study, the CUD group may have developed a greater fear-response (i.e., greater startle potentiation) to the countdown cue type, which is the time period that has the greatest likelihood of a shock occurring at the global task-level. Future work would benefit from examining whether fear learning and extinction influence startle reactivity, and the extent to which cannabis vs. non-using groups differ.

Current results may have useful treatment implications. Specifically, if allostatic load influences startle potentiation to threat-related stimuli in cannabis users, then startle blink magnitude may be used as a way to track CUD progression over time. Startle blink magnitude to unpredictable, but not predictable threat, appears malleable to cognitive-behavioral therapy (CBT) for fear-related disorders (Gorka et al., 2017); however, CBT that was successfully utilized to decrease alcohol consumption did not influence startle reactivity (Loeber et al., 2007). Considering general threat reactivity may be unique to chronic cannabis compared to other substance users, future work examining interventions that influence both phasic and sustained fear responses may be beneficial for chronic cannabis users who demonstrate startle potentiation to threat.

There are several limitations that must be addressed. First, participants in the cannabis-using group were required to refrain from cannabis use the day of their baseline lab visit. Considering all participants reported a history of cannabis-related withdrawal, it is possible that acute cannabis abstinence led to transient withdrawal symptoms during the baseline lab visit. Though cannabis deprivation has not been found to influence startle reactivity (Hefner et al., 2018) there is evidence that lower pain tolerance is related to greater withdrawal symptoms

(Manning et al., 2018). It is possible that acute withdrawal symptoms may have influenced acute tolerance for pain, subsequently influencing their maximum voltage for the NPU task; however, an exploratory RM-ANOVA revealed there was not a significant main effect for current withdrawal symptoms for condition, cue type, or their interaction (all ps >.27). Second, current study results have limited generalizability to several populations. Future studies would benefit from comparing startle reactivity in men and women who meet for severe CUD and likely exhibit a greater allostatic load (e.g., due to withdrawal history) compared to a non-using control group. Further, both groups in the current study were >80% White, limiting generalizability to other races. Third, the current study is cross-sectional in nature. As such, results preclude a causal explanation of the role of startle reactivity in severe CUD users. Allostatic theory may predict that chronic substance use leads to changes in startle reactivity, however greater startle reactivity may also be an endophenotype for harmful substance use (Gorka & Shankman et al., 2017). One way to examine whether allostatic load influences startle reactivity would be to examine whether abstinence-related upregulation of CB1 receptors or increased levels of anandamide result in changes to blink magnitude during the NPU task. Finally, current study groups were statistically matched on psychodiagnostics status; however, diagnoses included fear-related (i.e., panic-disorder) and anxious-misery (i.e., MDD, GAD) disorders, which may uniquely influence startle reactivity. Therefore, future study designs may benefit from examining fear- and anxious-misery-related disorders separately to more precisely understand how disorder type influences startle reactivity in severe CUD samples.

Results from the current study provide substantive, novel information about how a psychophysiological index of defensive threat response (i.e., startle reactivity) differs between a sample of women with severe CUD and non-cannabis using women matched on

psychopathology. Evidence in support for our initial hypothesis (i.e., the CUD group demonstrating amplified startle reactivity to unpredictable and predictable relative to no-threat conditions) partially replicates and extends previous literature examining the allostatic load model of addiction in cannabis users (Hefner et al., 2018). Overall, results suggest that greater allostatic load, presumably as a result of disordered cannabis use, influences startle reactivity to unpredictable and predictable threat during particular stimulus contexts (e.g., conditioned threat stimulus vs. contextual threat) beyond what would be expected from symptoms of psychopathology.

Table 1. Sample Descriptives.

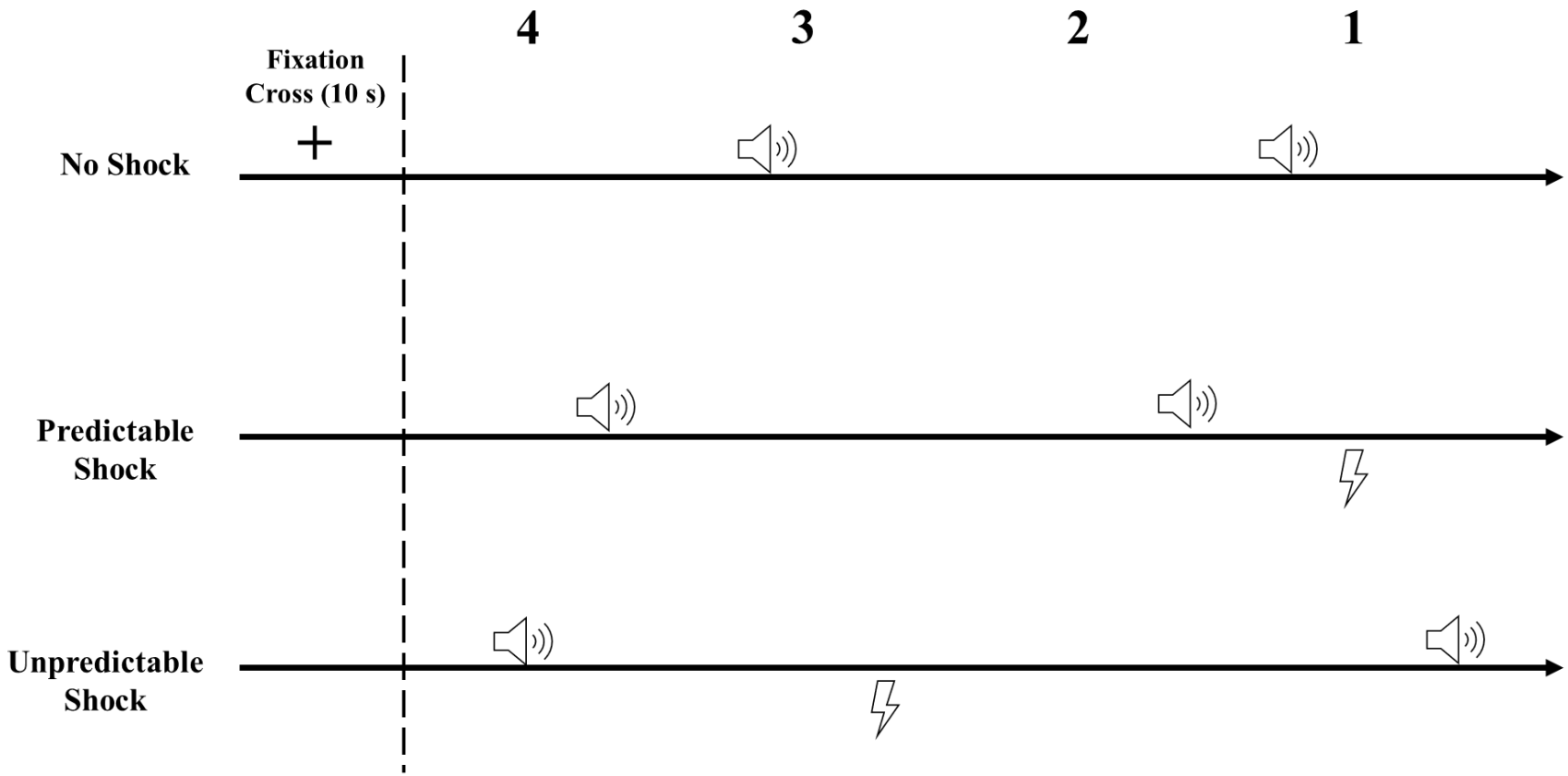
Measure	ICAE(*n*=20)	ICAE-C(*n*=20)		
Demographics	Mean (*SD*) or %		t or $\chi 2$	p (two-sided) or Fisher's exact
Age	21.75 (3.24)	19.15 (1.18)	3.37	**.003**
Race				
White	90%	85%		
Black	10%	0%		
Asian/Pacific Islander	0%	10%		
American Indian/Alaskan Native	0%	5%		
Hispanic	0%	5%		
Marital Status			2.11	.35
Single/Never Married	90%	100%		
Married	5%	0%		
Divorced	5%	0%		
Highest Level of Education			2.15	.34
High School Diploma	20%	25%		
Some College	70%	75%		
Bachelor's Degree	10%	0%		
Family Income			12.76	.12
$10,000 - $19,000	15%	0%		
$30,000 - $39,999	5%	0%		
$40,000 – 49,000	5%	0%		
$50,000 - $59,999	5%	5%		
$60,000 - $69,999	0%	10%		
$70,000 - $79,999	15%	10%		
$80,000 - $89,999	15%	5%		
$90,000 - $99,999	15%	5%		
$100,000 or more	25%	65%		
Currently Taking Birth Control			5.01	.05
Yes	60%	25%		
No	40%	75%		

Currently Menstruating			0.63	.70
Yes	25%	15%		
No	75%	85%		
Current Psychodiagnostic Status				
AUD	25%	10%	1.56	.41
Bipolar II, Current Depressive Episode	0%	0%		
Bipolar II, Current Hypomanic Episode	0%	0%		
Bipolar II, Current Euthymic Mood	5%	0%[a]	1.03	1.00
MDD Current, Single Episode	0%	0%		
MDD Current, Recurrent	10%	10%	0.00	1.00
Lifetime MDD Past Single Episode	20%	20%	0.00	1.00
Lifetime MDD, Recurrent Episodes	35%	35%	0.00	1.00
Panic*	10%	15%	0.23	1.00
Agoraphobia	5%	5%	0.00	1.00
Social Anxiety	10%	20%	0.78	.66
OCD	0%	0%		
PTSD	5%	0%[b]	1.03	1.00
Anorexia	0%	0%		
Bulimia	0%	0%		
Binge Eating	10%	0%[c]	2.11	.49
GAD	30%	35%	0.11	1.00
ASPD	5%	0%	1.03	1.00
No Diagnosis	20%	20%		
# of Diagnoses	1.70(*1.30*)	1.50(*1.23*)	0.52	.61
Startle Magnitude (uV)				
Habituation Period	138.32(*90.08*)	164.22(*106.83*)	-0.83	.41
No-Shock – CD	78.92(*59.46*)	102.47(*92.15*)	-0.96	.34
Predictable Shock – CD	110.02(*81.79*)	113.55(*100.25*)	-0.12	.90
Unpredictable Shock – CD	134.99(*80.87*)	135.10(*88.72*)	-0.004	.997
No-Shock – ISI	71.23(*60.24*)	88.12(*84.09*)	-0.73	.47
Predictable Shock – ISI	68.58(*61.93*)	96.24(*88.07*)	-1.15	.26
Unpredictable Shock – ISI	109.61(*71.03*)	124.60(*95.34*)	-0.56	.58
Max Shock Voltage (V)	67.37(*26.89*)	62.00(*16.34*)	0.76	.45
TLFB				
Past Month Alcohol Use Days	4.55(*3.33*)	1.65(*2.66*)	4.04	**0.004**
Past Month Nicotine Use Days	11.70(*14.13*)	2.15(6.02)	2.78	**0.01**
Cannabis use history				
Age at first cannabis use	16.40(*2.46*)	--		
Age of regular cannabis use onset	17.60(*2.04*)	--		
Total years of regular cannabis use	5.00(*3.61*)	--		

# of use days in past month	27.85(*5.00*)	--

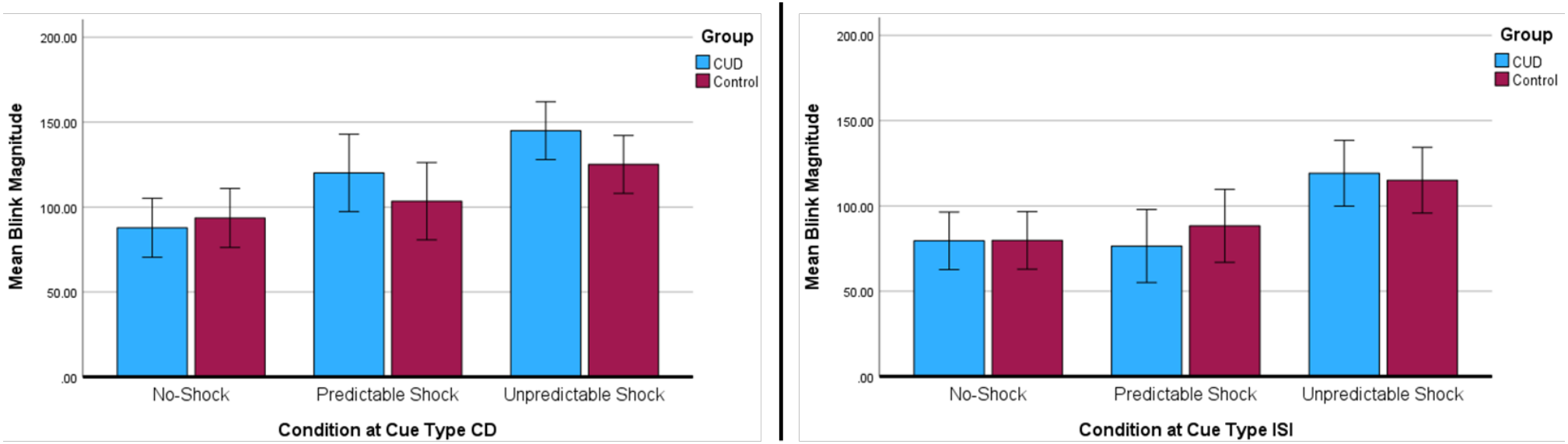
Note. AUD=Alcohol Use Disorder, MDD=Major Depressive Disorder, OCD=Obsessive Compulsive Disorder, PTSD= Posttraumatic Stress Disorder, GAD = Generalized Anxiety Disorder, ASPD=Antisocial Personality Disorder, CD=Countdown, ISI=Interstimulus Interval, TLFB=Timeline Follow-Back. [a]10% reported Past Hypomanic Symptoms, Current Euthymic, but did not meet for Bipolar 2. [b]5% were diagnosed with Acute Stress Disorder. [c]5% were diagnosed with Other Specified Feeding or Eating Disorder. *All participants met for Current Panic Disorder except for one ICAE-C participant, who met for Lifetime Panic Disorder.

Figure 1. No-Shock, Predictable Shock, and Unpredictable Shock (NPU) Task.



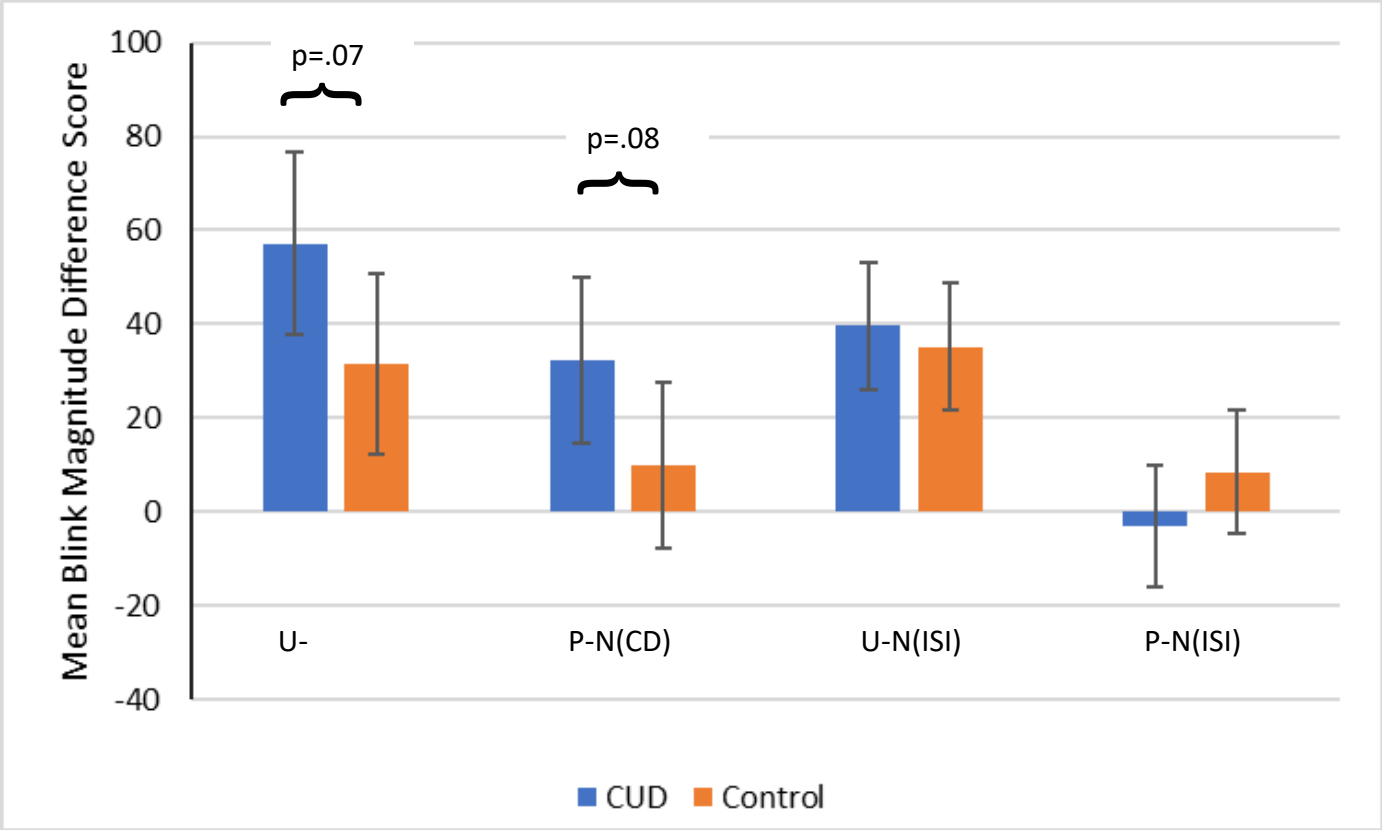
Note. All no-shock, predictable, and unpredictable shock blocks are 150 seconds and presented twice throughout the task. There are six, four-second countdowns per block, 10 startle probes, and six shocks per unpredictable/predictable block. Blocks are counterbalanced between participants (i.e., PNUPNU or UNPUNP). Predictable shocks are always delivered three seconds into countdown onset (i.e., at one).

Figure 2. Two-Panel Comparison of Mean Blink Magnitude by Condition and Cue Type Between Groups.



Note. N=No-Shock Condition, P=Predictable Shock Condition, U=Unpredictable Shock Condition, CD=Count Down cue type, ISI=Interstimulus Interval cue type. Error bars represent 95% Confidence Intervals. Estimated marginal means include habituation period as a covariate.

Figure 3. Startle Magnitude Potentiation Between Groups.



Note. U=Unpredictable Shock, N=No-Shock, P=Predictable Shock, CD=Countdown, ISI=Interstimulus Interval.

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