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# Impact of Childhood Maltreatment and Endocannabinoid Function on Posttraumatic Stress Symptoms in Traumatically-injured Adults

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by

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ABSTRACT

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The ECSS plays a crucial role in regulation of the stress response, is modulated by exposure to acute and chronic stressors, and shows potential as a biomarker for PTSD. Changes in ECSS function are apparent in adults with a history of childhood maltreatment. Further, childhood maltreatment is a well-established pre-trauma risk factor for development of PTSD following a traumatic event in adulthood. No study to date has examined the contribution of ECSS function to the relationship between childhood maltreatment and PTSD following a subsequent trauma in adulthood. The current study aimed to investigate the relationship between exposure to threat and deprivation experiences in childhood, circulating endocannabinoid concentrations and development of chronic PTSD following traumatic injury. To that end, $N = 46$ participants underwent study procedures acutely post-trauma and at a 6-month follow-up visit. Data collection included a blood draw and self-reported history of childhood maltreatment and PTSD symptoms associated with traumatic injury. Replicating previous findings, results show exposure to threat (in the form of physical, emotional, and/or sexual abuse), but not deprivation

(emotional and/or physical neglect), in childhood is predictive of chronic PTSD symptoms following a subsequent unrelated trauma in adulthood. The current study failed to replicate a small number of studies that have demonstrated a relationship between childhood maltreatment and circulating endocannabinoid levels in adulthood. While a mediational model including childhood threat experiences as predictor, AEA and 2-AG concentrations acutely post-trauma as mediators, and PTSD symptom severity 6-months post-traumatic injury as the outcome variable was significant overall ($p$=.003), only the direct paths for 2-AG levels to PTSD symptoms and childhood threat to PTSD symptoms were significant. These findings will be discussed within the context of the existing literature and important limitations of the current study.

To

my mother

my fiancé

and my dear friends

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**Posttraumatic stress disorder is prevalent, impairing and costly.**

National and global epidemiological surveys indicate that lifetime prevalence of exposure to a traumatic event is relatively common, with approximately 40-90% experiencing at least one traumatic event over their lifetime and approximately 30% experiencing four or more (Benjet et al., 2016; Kessler et al., 2017). The fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* defines a traumatic event as direct exposure to actual or threatened death, serious injury or sexual violence, or indirect exposure to such events (i.e., witnessing the event, learning of the event occurring to a loved one, repeated confrontation with aversive details of such events) (American Psychiatric Association, 2013). Substantial evidence suggests that exposure to trauma is associated with a variety of adverse mental and physical health outcomes (Keyes et al., 2013; Scott et al., 2013). Exposure to at least one traumatic event is required for diagnosis of posttraumatic stress disorder (PTSD), a psychiatric condition characterized by hyperarousal, intrusive reminders of the trauma, avoidance of trauma-related cues, and negative alterations to cognition and mood persisting longer than one-month post-trauma (American Psychiatric Association, 2013). Notably, despite the ubiquitous nature of trauma exposure, only a small subset of individuals who have experienced a traumatic event go on to develop PTSD, (approximately 20-30%) (Atwoli et al., 2015; Kessler et al., 2017). The type of trauma experienced significantly predicts PTSD onset and persistence with traumatic injury, especially in the context of interpersonal violence, conferring especially high PTSD burden (Alarcon et al., 2012; deRoon-Cassini et al., 2010; Kessler et al., 2017). Additionally, a range of pre-trauma factors that increase vulnerability for PTSD have been identified, including previous traumatic exposure, childhood adversity, chronic stressors, and psychiatric comorbidities (Kessler et al.,

2014; Smoller, 2016). Despite relatively low rates of PTSD onset following traumatic exposure, persistence of symptoms 6-months post-trauma is considered non-remitting PTSD. Non-remitting PTSD represents a significant public health problem, with high rates of functional impairment, poor mental and physical health outcomes, and high rates of psychiatric comorbidity, consuming the highest rate of resources for services (Benjet et al., 2016; Kessler et al., 2017). Thus, identification of biomarkers and increased understanding of the mechanisms through which pre-trauma factors promote risk or resilience for development of non-remitting PTSD following traumatic injury is imperative for facilitating effective treatments, mitigating the impact of traumatic exposure and decreasing likelihood of chronic disease (Bomyea et al., 2012; Horn et al., 2016; Michopoulos et al., 2015).

Substantial research has implicated impairments in extinction learning and hypothalamic-pituitary-adrenal axis dysregulation in development and maintenance of PTSD symptoms following trauma exposure (Lissek et al., 2005; Lissek & van Meurs, 2015; Yehuda et al., 1991). In the following sections, I will present evidence suggesting that traumatic exposure to interpersonal threat in childhood and endocannabinoid signaling system (ECSS) function may confer risk for PTSD via their influence on these mechanisms.

**Putative mechanisms underlying PTSD symptomatology.**

*Fear conditioning and extinction.* There is significant empirical support for conditioning models of PTSD, which implicate dysregulation of fear processing in its pathogenesis (Lissek & van Meurs, 2015). Specifically, individuals with PTSD show stronger acquisition of conditioned fear (i.e., heightened excitatory fear processes), and weaker extinction of conditioned fear (i.e., decreased inhibitory fear processes) (Lissek et al., 2005). While most trauma survivors (65-94%) experience post-traumatic stress symptoms (including the evocation of fear responses by stimuli

and situations that serve as “trauma reminders”) acutely post-trauma, only a small subset continue to retain symptoms one-month post-trauma and meet criteria for PTSD (Lissek & van Meurs, 2015). In those who go on to develop PTSD, it does not seem to be the acquisition of conditioned fear, but rather the retention of conditioned fear responding to reminders of the trauma that maintains symptoms (Lissek et al., 2005).

Additionally, studies investigating risk factors for the development of PTSD have consistently found increased risk in individuals with deficits in extinction learning that predate trauma exposure, suggesting that impaired extinction also represents a vulnerability factor (van Rooij et al., 2015). A distinguishing feature of PTSD among the anxiety disorders is that its pathogenesis is especially linked with aversive conditioning that takes place upon trauma exposure. Further, failure to extinguish associations between the trauma and everyday stimuli and situations and disinhibition of fear responses represent maintaining factors (McNally, 2007).

*Hypothalamic-pituitary-adrenal axis.* A well-established mechanism implicated in the pathophysiology of PTSD is dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (Yehuda et al., 1991). The HPA axis plays a critical role in the human neuroendocrine stress response. Under conditions of acute stress, corticotropin-releasing factor (CRF) is released from the hypothalamus, which thereby binds to CRF receptors on the anterior pituitary gland. When this occurs, adrenocorticotropic hormone (ACTH) is released and binds to receptors on the adrenal cortex stimulating adrenal release of cortisol. Release of cortisol persists for several hours after encountering a stressor, with blood concentrations of cortisol exerting negative feedback on subsequent hypothalamic release of CRF and pituitary release of ACTH. This negative feedback loop is critical in regulating the stress response and facilitating return to homeostasis (Yehuda et al., 1991, 2015). With repeated stress exposure, alterations to HPA axis

responsivity can occur such that the level of hypothalamic and pituitary sensitivity to blood concentrations of cortisol is either increased or decreased, resulting in dysregulation of the stress response. Under conditions of chronic stress, prolonged, elevated concentrations of glucocorticoids can have neurotoxic effects, impair cognitive and affective functions and lead to negative mental and physical health consequences (Yehuda et al., 2015). Thus, attenuation of the HPA stress response may represent a physiological adaptation to prolonged overactivation, serving as a compensatory mechanism to mitigate the harmful effects of chronically elevated cortisol and other glucocorticoids under conditions of chronic stress (Yehuda et al., 1991).

Alterations to HPA axis responsivity have been observed in major depressive disorder (MDD), which shares substantial symptom overlap and comorbidity with PTSD (Southwick, 1993). In MDD, these alterations are unidirectional in nature, characterized by HPA axis hyperactivation and decreased negative feedback sensitivity. Specifically, individuals with MDD show overproduction of cortisol, reduced number of glucocorticoid receptors and corticotropin-releasing factor (CRF) hypersecretion (Yehuda et al., 1991). Alternatively, many studies have provided evidence implicating underactivity of the HPA axis in PTSD, characterized by attenuated levels of basal cortisol and blunted cortisol reactivity (for review see Yehuda et al., 2005). A putative mechanism underlying blunted HPA axis activity in PTSD is heightened sensitivity to glucocorticoid negative feedback characterized by increased levels of CRF, elevated glucocorticoid receptor levels, increased glucocorticoid sensitivity and decreased inhibitory co-chaperones of glucocorticoid receptors (Baker et al., 2005; de Kloet et al., 2007; Matić et al., 2013). Notably, low levels of cortisol, elevated glucocorticoid receptor levels and decreased inhibition of glucocorticoid receptors in trauma survivors in the acute post-trauma period is associated with increased risk for development of PTSD (Mouthaan et al., 2014; van

Zuiden et al., 2012; Walsh et al., 2013). These effects have also been observed intergenerationally, with lower cortisol levels observed in both mothers and babies of mothers who were exposed to the World Trade Center collapse during pregnancy and went on to develop PTSD (Yehuda et al., 2005). However, studies investigating baseline cortisol levels as a biomarker of PTSD have been mixed, with some studies indicating hyperactivity similar to that seen in MDD and others finding no significant differences between PTSD and controls (Meewisse et al., 2007; Yehuda et al., 2015).

Discrepant findings of HPA dysregulation in PTSD may in part be due to anomalous sampling methods, diurnal rhythm of cortisol release and analyses that fail to account for sex differences in HPA reactivity (Freidenberg et al., 2010). Another fundamental challenge to understanding neuroendocrine dysregulation in PTSD includes difficulty disentangling the impact of the type and chronicity of TEs, the behavioral and clinical sequelae of PTSD itself, and vulnerability factors (e.g., genetic vulnerability, pre-traumatic stress history) on neuroendocrine function. It is possible that discrepant findings of HPA axis dysfunction in individuals with PTSD is due to the bidirectional modulation of HPA responses to stress dependent on stressor chronicity, severity, controllability and predictability and history of previous stress exposure (Yehuda et al., 1991).

**Impact of childhood adversity on stress-related symptoms and PTSD vulnerability**

Childhood adversity encompasses a wide range of events or experiences that pose a serious threat to a child's development and physical or psychological well-being (Glaser, 2000; Marinova & Maercker, 2015). Childhood adversity is a significant predictor of adult psychopathology broadly, and a robust body of literature demonstrates an association between a history of childhood adversity and development of PTSD in adulthood (Anda et al., 2006;

Brewin et al., 2000; Cabrera et al., 2007; Copeland et al., 2007; Kelleher et al., 2013; Kessler et al., 2018; McLaughlin & Lambert, 2017; Ozer et al., 2008). Indeed, rates of PTSD in individuals reporting two or more categories of adverse childhood events (ACEs) are significantly higher than unexposed controls, exceeding expected contribution of traumatic stressors occurring in adulthood (Cabrera et al., 2007; McLaughlin & Lambert, 2017; Pratchett & Yehuda, 2011). Further, exposure to traumatic events in childhood and history of anxiety-related psychopathology have been shown to contribute to individual differences in vulnerability to PTSD after a subsequent trauma (Copeland et al., 2007; DiGangi et al., 2013; Kessler et al., 2014, 2018; Schalinski et al., 2016) and diagnosis of PTSD in childhood has been shown to predict adult PTSD for unrelated subsequent traumas (Breslau & Peterson, 2010; Jones et al., 2013).

A substantial number of studies on associations between exposure to ACEs and neurodevelopmental outcomes utilize a cumulative risk approach, considering the total number of adversities experienced regardless of type. Due to the tendency for adversities to co-occur, this approach aims to collapse different categories of ACEs into one dimensional factor rather than attempt to disentangle their effects. Studies using this approach have established a dose-dependent relationship between number of ACEs experienced and negative health outcomes (Sheridan & McLaughlin, 2020). While studies utilizing a cumulative risk approach have robustly demonstrated relationships between ACEs and health outcomes, a significant limitation of this approach lies in its assumption of equifinality (multiple risk factors predicting a single outcome), which obscures potential specificity of effects (McLaughlin & Sheridan, 2016). In 2016, McLaughlin and Sheridan proposed a novel approach to conceptualizing childhood adversity along two distinct dimensions: experiences of threat and deprivation. The dimensional

model of adversity and psychopathology (DMAP) has been utilized in numerous studies since its inception, allowing greater specificity in our understanding of the impact of ACEs on health outcomes (McLaughlin & Sheridan, 2016). Early-life exposure to threat (physical, sexual, and emotional abuse and exposure to violence) and deprivation (physical and emotional neglect and separation from primary caregivers) have been shown to lead to differential outcomes via distinct pathways. Specifically, while exposure to threat is associated with aberrations in affective responding, it does not show an association with cognitive abilities. Alternatively, exposure to deprivation is associated with lower performance on measures of cognitive ability and not with aberrations in affective responding (Lambert et al., 2017; Machlin et al., 2019). Childhood exposure to threat is associated with information processing biases, disruptions in fear learning mechanisms, heightened affective responses to threat and difficulty disengaging from negatively-valenced stimuli (McLaughlin & Lambert, 2017). Further, exposure to threat-related ACEs is associated with increased risk for internalizing and externalizing psychopathology while deprivation shows associations with externalizing and not internalizing psychopathology (Miller et al., 2018; Milojevich et al., 2019). In adults, childhood threat, and not deprivation, experiences predict PTSD and negative affect (Greene et al., 2021; Guyon-Harris et al., 2021).

*Hypothalamic-pituitary-adrenal axis.* Exposure to adversity during critical neurodevelopmental periods in childhood puts individuals at risk for enduring alterations to neuroregulatory systems, increasing risk for psychopathology (Anda et al., 2006). In preclinical studies, exposure to early-life stress has been shown to result in behavioral alterations in fear responding and the endocrine-mediated stress response (McGowan, 2013; Tarullo & Gunnar, 2006). Numerous studies in humans have identified alterations to HPA-axis function at baseline and under stressful conditions in individuals exposed to childhood adversity broadly. However,

the directionality of these findings are mixed and differ based on sample age, timing, type and severity of adverse experiences, and concomitant internalizing and externalizing psychopathology (Tarullo & Gunnar, 2006). To further specify the effect of specific forms of childhood adversity on HPA-axis function in adolescence, Busso et al. (2017) utilized the DMAP theoretical model to test whether childhood experiences of deprivation and threat have differential impacts on HPA-axis development. Results indicated blunted HPA-axis response to psychological stress in adolescents exposed to interpersonal violence when accounting for environmental deprivation, and no relationship between HPA-axis reactivity and environmental deprivation when accounting for interpersonal violence. Further, HPA-axis reactivity to stress was shown to significantly mediate the association of violence exposure with internalizing and externalizing symptoms (Busso et al., 2017). Another study by Santa Ana et al. (2005) found significant differences in baseline and stress-induced cortisol levels that distinguished individuals with PTSD for adult versus childhood trauma and individuals with PTSD versus healthy controls. Results indicated that participants with PTSD, regardless of age at time of index trauma, had attenuated ACTH response compared to controls. Further, participants with childhood trauma showed lower cortisol at baseline and post stress-induction relative to participants with adult index trauma and controls. In participants with childhood index trauma, cortisol levels remained stable rather than decreasing over the 2-hour monitoring period, differentiating them from participants with adult index trauma and controls (Santa Ana et al., 2006). However, hypercortisolism has also been found in children with PTSD for sexual abuse (Tarullo & Gunnar, 2006) and adults exposed to childhood abuse (Bremner, 2006; Kindsvatter & Geroski, 2014; McGowan, 2013; Tyrka et al., 2012).

Importantly, there is a dearth of longitudinal studies documenting trajectories of HPA-axis function in individuals exposed to threat in childhood as they transition into adulthood. One longitudinal study of girls exposed to sexual abuse demonstrated hypercortisolism in childhood (indicated by elevated basal morning levels at age 11) and hypocortisolism in young adulthood (indicated by low basal levels at age 18) (De Bellis et al., 1994). The shift from hypercortisolism to hypocortisolism as children age is postulated to represent downregulation of the HPA-axis following sustained activation in response to chronic stress. Indeed, another study by Doom et al. (2014) demonstrated potential blunting of HPA-axis activity over time in children with chronically high cortisol levels, such that children with higher cortisol levels at initial assessment showed cortisol suppression over time (Doom et al., 2014).

PTSD is associated with dysregulation of the HPA axis, and downregulation of the HPA axis similar to that observed in adult PTSD is evident in individuals with a history of exposure to threatening experiences in childhood. Thus, it is possible that dysregulation of the HPA axis is an underlying mechanism by which childhood adversity confers risk for PTSD following traumatic exposure in adulthood. However, evidence to date in support of this putative mechanism primarily comes from animal studies and cross-sectional studies in humans, with limited longitudinal investigations. The link between HPA axis dysregulation in those with a history of childhood adversity and adult PTSD is blurred by within-group variability in cortisol levels dependent on severity, timing, and number of subtypes of trauma (Doom et al., 2014; Shea et al., 2005).

**The endocannabinoid signaling system.**

*Identification and characterization of human cannabinoid receptors. Cannabis sativa* is a flowering plant that has been used by humans for thousands of years for medicinal and

recreational purposes. The flowering tops and leaves of *C. sativa* secrete a resin containing phytochemicals termed cannabinoids, a group of terpenophenolic compounds that are known to produce euphoria, enhancement of sensory perception, reduced pain sensitivity, tachycardia, and impairments in concentration and memory (Ameri, 1999). In 1964, Gaoni & Mechoulam isolated and identified the psychoactive component of *C. sativa*, $\Delta^9$-tetrahydrocannabinol (THC). Subsequent studies on the bioactive effects of synthetic structural analogs of THC, levo-nantradol and [$^3$H]CP55940, led to discovery and characterization of the endogenous cannabinoid signaling system (ECSS) in rodents and humans (Hillard, 2015). These seminal observations laid the foundation for research focused on understanding the pharmacological and biochemical properties of cannabinoids and the mechanisms by which they impact physiology and behavior. Additionally, given the apparent role of ECSS dysregulation in pain, psychiatric, neurodegenerative and inflammation-related disorders, investigation into potential therapies that act on the ECSS is well-underway (Freund et al., 2003; Hillard, 2015; R. J. McLaughlin et al., 2014; Piomelli, 2003).

*Cannabinoid receptors.* Inter-neuronal communication in the brain relies on neurotransmission at synaptic sites. The ECSS operates at synapses in a retrograde manner, with receptors located on axon terminals of a presynaptic cell. The cannabinoid receptors, CB1 and CB2, are inhibitory G-protein coupled receptors (GCPRs); upon extracellular binding with a ligand, GCPRs activate G proteins intracellularly, thereby triggering production of secondary messengers which serve to induce a cellular response (deRoon-Cassini et al., 2020; Mackie, 2008; R. J. McLaughlin et al., 2014; Piomelli, 2003). Throughout the body, GPCRs are crucial in initiating signaling pathways in inflammation and neurotransmission (Hillard, 2015). CB1 receptors are the most abundant class of GCPRs in the brain, with widespread expression in the

forebrain, basal ganglia and limbic system, while CB2 receptors are found in immune system cells, including brain microglia. In particular, the CB1 receptor signaling system plays a ubiquitous role in regulation of neurotransmission (Hillard, 2015).

Distributed on both glutamatergic and GABAergic neurons, which act as major excitatory and inhibitory neurotransmitters, respectively, CB1 receptors inhibit neurotransmitter release to modulate the balance of excitation and inhibition within a given neural circuit (deRoon-Cassini et al., 2020; R. J. McLaughlin et al., 2014). CB1 receptors located on glutamatergic and GABAergic neurons have been shown to have functionally dissociable roles, demonstrated by the biphasic effect of low- and high-dose cannabinoid exposure on anxiety-like responding. Low-dose cannabinoid exposure has been shown to have an anxiolytic effect, mediated by CB1 receptors on cortical glutamatergic synaptic terminals. Alternatively, high dose cannabinoid exposure produces an anxiogenic effect mediated by CB1 receptors on inhibitory GABAergic neurons (Lutz et al., 2015; R. J. McLaughlin et al., 2014).

*Endogenous cannabinoids.* The two most well-studied naturally-occurring endogenous ligands for cannabinoid receptors, termed endocannabinoids, are *N*-arachidonoylethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) (Hillard, 2015, 2018). Both AEA and 2-AG act as agonists on cannabinoid receptors and belong to a family of *N*-acylethanolamines (NAEs) that also includes non-endocannabinoid *N*-palmitoylethanolamine (PEA), *N*-oleoylethanolmine (OEA), *N*-stearoylethanolamine (SEA) and *N*-docosahexaenoylethanolamine (DEA). These related non-endocannabinoid NEAs are concomitantly produced with AEA and share similar metabolic pathways (Hillard, 2015; Tsuboi et al., 2018). Unlike traditional neurotransmitters which are stored in vesicles, both AEA and 2-AG are synthesized on-demand in postsynaptic cells following synaptic membrane depolarization. When released into the

synapse, AEA and 2-AG travel in a retrograde manner to activate CB1 receptors located in the presynaptic membrane, hyperpolarizing the membrane and inhibiting neurotransmitter release (Hillard, 2015; McLaughlin et al., 2014).

Importantly, AEA and 2-AG have distinct pharmacokinetic properties in terms of binding affinity, distribution, and metabolism. 2-AG, a high-abundance 2-monoacyl glycerol, is biosynthesized from inositol phospholipids via hydrolysis by phospholipase C and diacylglycerol lipase. Degradation of 2-AG occurs via hydrolysis to arachidonic acid and glycerol catalyzed by monoacylglycerol lipase (MAGL) and fatty acid amid hydrolase (FAAH). Compared to the MAGL-dependent pathway, FAAH-dependent degradation of 2-AG is considered to be less crucial *in vivo.* Notably, MAGL-dependent generation of arachidonic acid from 2-AG has been shown to produce prostaglandins, which promote neuroinflammation (Lutz et al., 2015; Tsuboi et al., 2018).

Alternatively, AEA is a found in relatively low abundance in tissues throughout the body and is thought to be biosynthesized via multiple redundant pathways including generation from *N*-arachidonoyl phosphatidylethanolamine (NAPE) via cleavage by phospholipase D (Maccarrone, 2017). AEA and related *N*-acylethanolamines are degraded through FAAH-dependent hydrolysis into free fatty acids and ethanolamine (Tsuboi et al., 2018; Maccarrone, 2017). FAAH plays a critical role in the degradation of *N*-acylethanolamines, such that specific FAAH inhibitors are under investigation for therapeutic use against pain, affective and anxiety disorders. Specifically, FAAH inhibitors function to halt AEA degradation thereby increasing tissue levels of AEA and their action on cannabinoid receptors. Combined FAAH and MAGL inhibitors have also been developed to increase both AEA and 2-AG levels to mimic pharmacological effects of a CB1 receptor agonist *in vivo* (Tsuboi et al., 2018; Lutz et al., 2018).

Differences in binding affinity for both CB1 and CB2 receptors exist between AEA and 2-AG, producing distinct signaling patterns. AEA shows high affinity for CB1 receptors and acts as a partial agonist. AEA binding fails to activate G protein signaling in CB2 receptors, suggesting that it is a weak partial agonist of this receptor type (Hillard, 2015). Although 2-AG shows relatively lower affinity for CB1 receptors compared to AEA, it acts as a full agonist on both CB1 and CB2 receptors. AEA and 2-AG also show differences in distribution, such that 2-AG levels in tissue are generally hundreds to thousands of times higher than those of AEA. Thus, 2-AG is thought to play a more critical role in endocannabinoid system signaling *in vivo* compared to AEA. Given these distinct signaling patterns, 2-AG binding produces a robust intracellular response, while AEA binding results in tonic and mild stimulation. Thus, 2-AG may function to mobilize rapid and robust modulation of activity-induced synaptic plasticity while AEA promotes fine-tuning and/or maintenance of the ECSS (Tsuboi et l., 2018; McLaughlin et al., 2014). Importantly, although endocannabinoids are produced in a transient fashion, they play a major role in both short-term (spike time-dependent plasticity, depolarization- and metabotropic-induced suppression of inhibition/excitation) and long-term (long-term depression) neuronal plasticity and their effects can be long-lasting (Mackie, 2008).

*Endocannabinoid system and emotional responding.* Distribution of CB1 receptors is highly concentrated in neural circuits involved in affective responding. Particularly high-density CB1 receptor distribution is evident in the cingulate gyrus, frontal cortex, hippocampus, cerebellum and basal ganglia, with moderate CB1 receptor densities in the basal forebrain, amygdala, nucleus accumbens, periaqueductal gray and hypothalamus. Alternatively, CB1 receptors are relatively sparsely distributed in the midbrain, pons and medulla, and particularly

low distribution is found in primary motor and sensory cortices (Hillard, 2015; McLaughlin et al., 2014; Mackie, 2005; Lutz et al., 2018).

As their pattern of distribution suggests, CB1 receptors modulate inhibition and excitation in neural circuits critical to emotional responding. Thus, one of the main functions of the ECSS may be to act as a regulatory buffer system for the stress response (deRoon-Cassini et al., 2020; Finn et al., 2012). Indeed, substantial evidence demonstrates biphasic effects of cannabinoid receptor agonists on anxiety-related behavior in a dose- and context-dependent manner (Finn et al., 2012). Specifically, CB1 receptors located in the medial prefrontal cortex (mPFC), basolateral amygdala (BLA), bed nucleus of the stria terminalis (BNST), hippocampus and dorsal periaqueductal grey (PAG) are implicated in cannabinoid-mediated modulation of anxiety and defensive behavior (Finn et al., 2012; Morena et al., 2016). Notably, due to the plasticity of the ECSS and its action on both excitatory (glutamatergic) and inhibitory (GABAergic) neurons, differential effects of stress on ECSS expression across brain regions is observed (Finn et al., 2012; Lutz et al., 2018).

The anxiolytic effects of FAAH inhibitors has been well-demonstrated in animal models, with systemic administration reducing anxiety-like behavior under aversive conditions (Haller et al., 2009). However, some discrepant findings exist, such that FAAH inhibitors failed to have anxiolytic effects under certain conditions. Importantly, FAAH inhibitors promote AEA signaling only when and where it is already occurring and do not induce it. In support of the potential for FAAH inhibitors to produce anxiolytic effects, robust anxiolytic effects under exposure to highly aversive, but not mildly stressful, conditions have been found (Haller et al., 2009).

ECSS modulation of anxiety-like behavior in rodents is dependent on levels of emotional arousal in the experimental context. Numerous studies support state-dependent modulation of endocannabinoid signaling through systemic administration of AEA and 2-AG hydrolysis (MAGL and FAAH) inhibitors. Specially, global pharmacologically-induced elevations of AEA and 2-AG have been shown to decrease anxiety under conditions of high emotional arousal/stress and not under conditions of low arousal/stress (Aliczki et al., 2012; Haller et al., 2009; Naidu et al., 2007; Sciolino et al., 2011). Interestingly, this pattern of results is different when manipulations of endocannabinoid signaling are localized to the BLA rather than systemic. Notably, pharmacologically-induced elevations of AEA and 2-AG via hydrolysis inhibitors administered locally in the BLA resulted in attenuation of anxiety-like behavior under conditions of low emotional arousal but not under conditions of high emotional arousal (Morena et al., 2016).

In the prefrontal cortex (PFC), CB1 receptors are densely deposited on inhibitory GABA terminals. Importantly, the PFC plays an important role in emotional learning, specifically by engaging appropriate executive functioning processes during exposure to emotionally salient stimuli via the cortico-amygdalar pathway. CB1 receptors appear particularly important for maintaining balance along the cortico-amygdalar pathway through modulation of excitation/inhibition. Compromised connectivity between the PFC and basolateral amygdala is evident under conditions of chronic stress and is associated with vulnerability for development of psychopathology (McLaughlin et al., 2014).

Impaired ECSS function is implicated in numerous anxiety and affective disorders and significantly impacts stress reactivity. Indeed, CB1 knockout mice display anxiogenic- and depressive-like phenotypes as well as profound alterations in adrenocortical activity. Similarly,

pharmacological blockade of CB1 receptors induces anxiety in rats while pharmacological inhibition of AEA hydrolysis has an anxiolytic effect (Lutz et al., 2015; M. Viveros et al., 2005). In humans, a functional *FAAH* gene variant (rs324420) that reduces FAAH expression is associated with significantly lower self-reported stress reactivity ($t = 2.39$, $p > 0.05$) (Gunduz-Cinar et al., 2013).

*Role in fear conditioning and extinction.* Evidence from preclinical studies suggests that synaptic plasticity within the amygdala is crucial in acquisition, storage and extinction of aversive memories and is critically mediated by the ECSS (Viveros et al., 2007). An eyeblink conditioning study by Steinmetz & Freeman (2010) demonstrated dose-dependent impairments in acquisition following systemic administration of a CB1 agonist and a CB1 antagonist, with CB1 agonist-injected rats showing greatest magnitude of impairment. Further, dose-dependent impairments in the conditioned eyeblink response amplitude and timing were apparent in rats injected with a CB1 agonist and not those injected with a CB1 antagonist. This suggests that depolarization-induced suppression of both excitation and inhibition via CB1 signaling can impair fear learning (Steinmetz & Freeman, 2010).

In rats, endocannabinoids have been found to facilitate extinction of aversive memories through inhibition of local inhibitory networks of the amygdala and increased endocannabinoid tone enhances extinction of conditioned fear responding (Finn et al., 2012; Marsicano et al., 2002). During an extinction protocol, endocannabinoid levels are elevated within the basolateral amygdala (BLA), a region known to control extinction of aversive memories (Marsicano et al., 2002). Systemic administration of a selective FAAH-inhibitor (increasing brain AEA levels) in rats has been shown to facilitate fear extinction via the amygdala and enhance amygdala-specific synaptic plasticity (Gunduz-Cinar et al., 2013). Further, CB1 knock-out mice show impaired

short- and long-term extinction in fear conditioning tasks with no deficits in memory acquisition or consolidation, and pharmacological blockade of CB1 receptors in mice and rats results in dose-dependent impairments in extinction of aversive memories (Marsciano et al., 2002).

Interestingly, CB1 knock-out mice and rats administered a FAAH inhibitor do not show deficits in extinction of appetitively-reinforced memories, suggesting that CB1 receptors play a crucial role in extinction of aversive memories specifically (Hölter et al., 2005; Niyuhire et al., 2007). Administration of a FAAH inhibitor has been shown to enhance extinction learning. This AEA-mediated enhancement is dose-dependently blocked by CB1 receptor blockade, suggesting involvement of CB1 receptors (Chhatwal et al., 2005). Studies administering FAAH inhibitor and CB1 receptor agonists have demonstrated blocked reinstatement of fear following extinction (Lin et al., 2006; Chhatwal et al., 2005). In humans, a functional *FAAH* gene variant (rs324420) that reduces FAAH expression has been shown to modulate amygdala-driven habituation to threat, such that individuals with the variant demonstrated greater habituation of amygdala activation to threatening faces (Gunduz-Cinar et al., 2013).

Aberrations in fear inhibition are observed in numerous psychiatric disorders, including phobias and PTSD (Viveros et al., 2007). Based on findings of both impaired extinction and habituation of the fear response in CB1 knock-out mice, a mouse model of PTSD has been proposed such that CB1 receptors impact extinction through facilitation of non-associative learning (habituation) (Siegmund & Wotjak, 2007).

*Endocannabinoid modulation of the HPA axis*. In preclinical studies, the ECSS has been shown to exert influence over the stress response via regulation of HPA axis activity. CB1 receptors are densely distributed in brain regions involved in HPA axis regulation, including the hippocampus, amygdala and PFC. To a lesser degree, CB1 receptors are also expressed in

subcortical regions such as the bed nucleus of the stria terminalis (BNST) and paraventricular nucleus (PVN) of the hypothalamus. CB1 receptor mRNA co-localizes with CRF mRNA in the PVN, amygdala, PFC and BNST, and it is suspected that presynaptic activation of CB1 receptors serves to regulate neuronal release of CRF (Micale & Drago, 2018). Under baseline conditions, the ECSS been shown to tonically inhibit HPA axis activity via CB1 receptors in the hypothalamus that inhibit CRF signaling. Under conditions of acute stress, endocannabinoid signaling is reduced to allow disinhibition of the HPA axis and mounting of the stress response. Under conditions of chronic stress, endocannabinoid levels increase to inhibit prolonged HPA axis activation and restore homeostasis (Viveros et al., 2007). Administration of a CB1 receptor antagonist has been shown to increase serum corticosteroid concentrations at baseline and potentiate HPA axis activation under stressful conditions. Alternatively, pretreatment of mice with a CB1 receptor agonist or FAAH inhibitor significantly decreases or eliminates stress-induced corticosteroid release. Further, stress-induced corticosteroid release is associated with decreased 2-AG levels in the hypothalamus, whereby attenuation of the adrenocortical response is associated with increased 2-AG levels (Akirav, 2011; Hill et al., 2009).

The interaction between the ECSS and HPA axis is not unidirectional, but rather includes plasticity of the ECSS mediated by stress and HPA responsivity. Chronic stress exposure alters the ECSS throughout the brain, resulting in downregulation of CB1 receptor signaling in brain regions involved in affective processing, including the hypothalamus, amygdala, dorsal raphe nucleus, PFC, nucleus accumbens, striatum and hippocampus. In the hypothalamus, striatum and hippocampus, chronic stress is associated with decreased CB1 receptor density, while in the PFC chronic stress increases CB1 receptor mRNA expression but reduces CB1 receptor responsivity on GABAergic neurons. In the amygdala, chronic stress increases FAAH activity, thereby

decreasing AEA concentrations and CB1 receptor signaling (Viveros et al., 2005). Enzymatic processes involved in synthesis and degradation of endocannabinoids are modulated by acute and chronic stress exposure, altering endocannabinoid concentrations and CB1 receptor signaling (deRoon-Cassini et al., 2020). Downregulation of the ECSS following exposure to chronic stress may contribute to poor adaptation and excessive stress response (deRoon-Cassini et al., 2020).

**Endocannabinoid signaling system implicated in pathophysiology of PTSD.**

The ECSS plays a crucial role in the stress response and is altered by exposure to stress and trauma (Hillard, 2014). Alterations to the ECSS are implicated in the development and maintenance of stress-related psychopathology and are considered to be long-lasting (Bluett et al., 2017; Micale & Drago, 2018). Thus, pharmacological manipulation of the ECSS has been investigated as a possible target in treatment of PTSD symptoms (Akirav, 2013; Bassir Nia et al., 2019; Berardi et al., 2016; Hindocha et al., 2020; Horn et al., 2016; Korem et al., 2016; Neumeister et al., 2015; Papini et al., 2015; Pinna, 2018; Trezza & Campolongo, 2013).

During and in the immediate aftermath of a traumatic event, tonic AEA signaling is attenuated to disinhibit HPA activity and mount a stress response. In preclinical studies, rapid attenuation of AEA under conditions of acute stress has been observed in the amygdala and hippocampus but not in the PFC, suggesting regional specificity. Alternatively, tissue concentrations of 2-AG in the hypothalamus, hippocampus and PFC, but not in the amygdala, have been shown to increase following acute stress (Bassir Nia et al., 2019). 2-AG is thought to play a crucial role in stress resilience, such that pharmacological increase in 2-AG enhances resilience to trauma in previously vulnerable mice. Alternatively, systemic depletion of 2-AG or CB1 receptor blockade increases vulnerability to development of PTSD-like symptoms in previously resilient mice. Resilience to a traumatic stressor is associated with increased phasic 2-

AG mediated inhibition of glutamatergic neurons in the hippocampus and amygdala. Depletion of 2-AG in the amygdala has been shown to impair adaptation to repeated stress (Bluett et al., 2017).

In the human literature, an increasing number of studies have investigated the role of alterations to the ECSS in individuals with PTSD. However, most of these studies rely on peripheral measures of endocannabinoid ligands (i.e., plasma and hair concentrations) and results have been largely inconsistent. A study by Hill et al. (2013) investigated the relationship between PTSD symptoms and circulating endocannabinoids in individuals exposed to the World Trade Center attack. Findings indicated a negative association between circulating AEA levels and re-experiencing PTSD symptoms. This finding is consistent with preclinical data suggesting that decreased AEA levels promote retention of aversive memories. Further, those with non-remitting PTSD had lower circulating levels of 2-AG compared to trauma-exposed controls, suggesting that deficient endocannabinoid signaling may promote HPA dysregulation and be associated with PTSD following a traumatic event (Hill et al., 2013). Similarly, a positron emission tomography (PET) study by Neumeister et al. (2013) found significantly reduced AEA concentrations and increased CB1 receptor density in participants with PTSD relative to trauma-exposed controls and unexposed controls. Upregulation of CB1 receptors and decreased AEA levels are suggestive of decreased AEA tone in PTSD (Neumeister, 2013). Lower hair concentrations of related NAEs have also been found in patients with PTSD relative to controls, as well as a strong negative association between NAE levels and PTSD symptom severity (Wilker et al., 2016).

On the other hand, a study by Hauer et al. (2013) found higher plasma concentrations of AEA, 2-AG and related NAEs in individuals with PTSD compared to unexposed controls.

Additionally, individuals with PTSD showed higher 2-AG and related NAE concentrations compared to trauma-exposed individuals without PTSD (Hauer et al., 2013). A recent investigation by deRoon-Cassini et al. (2022), which was notably the first to investigate the relationship between acute serum endocannabinoid concentrations and chronic PTSD, found significantly higher circulating AEA concentrations acutely post-trauma in individuals diagnosed with PTSD 6-8 months post-trauma (deRoon-Cassini et al., 2022). In the same study, circulating 2-AG concentrations acutely post-trauma were found to be significantly positively associated with PTSD symptom severity at follow-up, although this was specific to participants from racially/ethnically-minoritized groups. Interestingly, in the same subset of participants AEA concentrations and PTSD symptoms severity were negatively correlated acutely post-trauma. Additionally, correlational analyses stratified by sex found a positive relationship between AEA concentrations and PTSD symptom severity at follow-up in female participants (deRoon-Cassini et al., 2022). Notably, both preclinical and clinical studies have observed sex differences in CB1 receptor regulation, with up-regulation of CB1 receptors observed more robustly in those assigned female at birth, particularly in the context of PTSD (Neumeister, 2013).

The above findings point to alterations in endocannabinoid signaling that distinguish individuals with PTSD from trauma-exposed individuals without PTSD and those who are unexposed. However, due to discrepant findings it is difficult to ascertain under which conditions, for whom, and in which direction alterations to endocannabinoid concentrations, both acutely post-trauma and at long-term follow-up, represent a risk factor for development of non-remitting PTSD. Notably, circulating endocannabinoid concentrations are influenced by circadian changes, food intake, and time since trauma, which may pose some challenges for their interpretation (Hillard, 2018). Additionally, lack of differentiation between timing (i.e.,

childhood versus adulthood), chronicity and severity of lifetime trauma exposure may occlude some effects (Ballard et al., 2015; Benjet et al., 2016; Ehlert, 2013; Kessler et al., 2018).

**Early-life stress associated with alterations to endocannabinoid signaling system.**

Early-life stress can affect critical neurodevelopmental periods, leading to dysregulation of homeostatic systems in adulthood. Notably, mechanisms underlying stress response, including the HPA axis, appear particularly impacted by exposure to early-life stress (Bremner, 1999; Galve-Roperh et al., 2009; McGowan, 2013; Moussa-Tooks et al., 2020).

Preclinical studies demonstrate that regulation of the HPA axis, which triggers release of cortisol from the adrenal cortex, relies critically on the ECSS. Chronic life stress has been shown to alter hippocampal endocannabinoid system function by increasing 2-AG and thereby increasing cortisol production and release (Morena et al., 2016). Rodent studies also support altered expression of endocannabinoid-related genes as a consequence of early-life stress (i.e., maternal deprivation). Marco et al. (2014), found increased genetic expression of genes encoding for CB1 and CB2 receptors, and major synthesizing enzymes in the frontal cortex in male rats and hippocampus in female rats. This suggests that the endocannabinoid system is sensitive to early-life stress, with sex- and region-dependent effects on gene expression starting in adolescence (Marco et al., 2014). Vangopoulou et al. (2018) found that neonatal handling, an experimental model of early-life stress associated with resilience to stress later in life, resulted in alterations to cannabinoid receptor mRNA and binding levels. Specifically, neonatal handling interfered with developmental trajectories of CB1 receptor mRNA levels in the striatum and amygdala nuclei. Adult handled rats showed reduced binding levels in the prefrontal cortex, striatum, nACC and basolateral amygdala, while binding levels in prefrontal cortex of adolescent handled rats were increased (Vangopoulou et al., 2018). While the ECSS has been implicated in

stress adaptation and affective regulation in animal studies, few human studies have examined the relationship between ECSS function and childhood adversity.

*Link between childhood adversity and alterations in human ECSS.* Clinical studies indicate that, while decreased levels of endocannabinoid ligands and increased levels of cannabinoid receptors are generally observed in individuals with adult trauma, decreased levels of both endocannabinoid ligands and receptors are observed in individuals with history of childhood trauma. This suggests differential impact of exposure to a traumatic event dependent on age of occurrence (Bassir Nia et al., 2019). Importantly, some evidence suggests that neurodevelopmental changes stemming from exposure to early-life stress may not be detectable until adulthood due to rapid changes in neural circuitry throughout childhood. This underscores the importance of studying long-term effects of early-life stress on neural systems (Baker et al., 2005; Bonne et al., 2001). These long-term effects were observed in a study by Koenig et al. (2018b), which found plasma concentrations of a compound associated with AEA oxidation to be increased in postpartum women with exposure to childhood maltreatment broadly (Koenig et al., 2018b). The effects of early-life stress on the ECSS have also been observed intergenerationally. Another study by Koenig et al. (2018a) showed higher endocannabinoid-related NAE concentrations in the hair of third-trimester pregnant women exposed to childhood maltreatment compared to those who were unexposed. With greater maternal childhood maltreatment severity (measured as a cumulative score collapsed across type), lower maternal SEA levels and higher neonatal OEA levels were observed. Notably, 1-AG and OEA are considered endocannabinoid-related bioactive lipids, are chemically similar to AEA and potentiate effects of AEA. This suggests that altered endocannabinoid levels are apparent in third-trimester pregnant women with a history of childhood maltreatment as well as their

developing fetus. This highlights potential intergenerational effects on the endocannabinoid signaling system resulting from maternal exposure to childhood maltreatment (Koenig et al., 2018a).

*Link between childhood maltreatment-related alterations to ECSS and PTSD.* Cumulative experiences of childhood adversity is a well-established risk factor for adult psychopathology broadly (Agrawal et al., 2012; Appiah-Kusi et al., 2020; Hornung & Heim, 2014; Lazary et al., 2016; Mello et al., 2009). Specifically, threat-related maltreatment (i.e., sexual, physical and emotional abuse) shows robust relationships with fear dysregulation and PTSD (Greene et al., 2021; Machlin et al., 2019; McLaughlin et al., 2015; McLaughlin & Lambert, 2017). Stress-induced alterations to the ECSS during neurodevelopment may represent a mechanism through which childhood threat experiences confer risk for development of PTSD in adulthood.

Borderline personality disorder (BPD) and complex posttraumatic stress disorder (c-PTSD) are highly associated with interpersonal violence during childhood and adolescence. A study by Schaefer et al. (2014) analyzed serum levels of AEA and 2-AG and related fatty acid ethanolamides (FAEs) in individuals with BPD, complex PTSD (related to childhood sexual abuse) and healthy controls. Compared to healthy controls, serum levels of AEA were significantly elevated in individuals with BPD, while OEA was significantly elevated in individuals with complex PTSD. Compared to individuals with complex PTSD, individuals with BPD showed significantly elevated 2-AG serum levels. Notably, 10 out of 26 participants meeting criteria for BPD met criteria for co-occurring complex PTSD in this sample. An independent analysis showed no difference between subjects with comorbid BPD and complex PTSD and those with BPD alone (Schaefer et al., 2014).

## Current study

The ECSS plays a crucial role in regulation of the stress response, is modulated by exposure to acute and chronic stressors, and shows potential as a biomarker for PTSD. Changes in ECSS function are apparent in adults with a history of childhood maltreatment (Croissant et al., 2020; Koenig, Gao, et al., 2018). Further, childhood maltreatment is a well-established pre-trauma risk factor for development of PTSD following a traumatic event in adulthood (Mello et al., 2009). No study to date has examined the contribution of ECSS function to the relationship between childhood maltreatment and PTSD following a subsequent trauma in adulthood. The current study aimed to investigate the relationship between exposure to threat and deprivation experiences in childhood, circulating endocannabinoid concentrations and development of chronic PTSD following traumatic injury. To that end, participants underwent study procedures acutely post-trauma and at a 6-month follow-up visit. Data collection included a blood draw and questionnaires assessing history of childhood maltreatment and PTSD symptoms associated with traumatic injury.

Aim 1: Investigate the association between self-reported threat- (emotional, physical and sexual abuse) and deprivation-related (emotional and physical neglect) childhood experiences and circulating endocannabinoid (2-AG, AEA) levels (a) acutely post-traumatic injury and (b) 6 months post-traumatic injury.

Hypothesis: (1a) Based on previous findings (deRoon-Cassini et al., 2022; Koenig et al., 2018; McLaughlin & Sheridan, 2016) self-reported history of childhood threat experiences (and not deprivation experiences) is expected to be positively associated with 2-AG, AEA levels in acute post-traumatic period and (1b) 6 months post-trauma.

Aim 2: Examine whether the relationship between self-reported childhood maltreatment and PTSD symptom severity (PCL-5) 6 months post-traumatic injury is mediated by circulating endocannabinoid levels (2-AG, AEA) in acute post-trauma period.

Hypothesis: It is expected that circulating 2-AG and AEA levels acutely post-trauma will partially mediate the relationship between self-reported childhood threat experiences (and not deprivation experiences) and PTSD symptoms 6 months post-trauma, such that self-reported history of childhood maltreatment will be associated with increased PTSD symptoms when circulating 2-AG and AEA levels are increased in the acute post-trauma period.

## Method

### *Participants*

The current study utilized data collected as part of a larger R56 study at the Medical College of Wisconsin. Participants were adults recruited from the inpatient trauma service of Froedtert Hospital, a Level 1 trauma center, following hospitalization for traumatic injury. Initial eligibility was determined using a trauma service census. Exclusion criteria included: 1) younger than 18 years of age, 2) traumatic brain injury resulting in peritraumatic amnesia for greater than 30 minutes, 3) inability to communicate due to injury, 4) Glasgow coma score of less than 13 on admission. A total of 46 participants were included in the current study. Data was collected from 93 participants at the 6-month post-trauma follow-up visit. To date, the blood assays of 42 participants are still being processed. Of 51 participants with processed blood assays, two were excluded due to incomplete data. Three additional participants were excluded due to extreme data values (±3 *SD* from mean). Sample characteristics for the current study are presented in Table 1.

| | *Mean (SD) / n (%)* |
|---|---|
| Gender | |
| Female | 13 (28%) |
| Male | 33 (72%) |
| Age | 38.53 (*16.57*) |
| Race/Ethnicity | |
| American Indian/Alaska Native | 1 (2%) |
| Asian | 1 (2%) |
| Black or African-American | 35 (76%) |
| Hispanic or Latino/a/x | 3 (7%) |
| White | 6 (13%) |
| Mechanism of Injury | |
| Motor vehicle crash | 23 (50%) |
| Gun shot | 10 (22%) |
| Stab | 2 (4%) |
| Fall | 2 (4%) |
| Pedestrian struck | 1 (2%) |
| Motorcycle crash | 6 (13%) |
| Other | 2 (4%) |
| PCL-5 | |
| Total score (baseline) | 21.15 *(19.22)* |
| Total score (6 months) | 28.30 (*23.90*) |
| CTQ (6 months) | |
| Emotional abuse (Y/N) | 22 (48%) / 24 (52%) |
| Subscale total | 8.83 *(4.57)* |
| Physical abuse (Y/N) | 25 (54%) / 21 (46%) |
| Subscale total | 8.20 *(2.91)* |
| Sexual abuse (Y/N) | 9 (20%) / 37 (80%) |
| Subscale total | 6.43 *(3.93)* |
| Emotional neglect (Y/N) | 14 (30%) / 32 (70%) |
| Subscale total | 9.02 *(4.37)* |
| Physical neglect (Y/N) | 13 (28%) / 33 (72%) |
| Subscale total | 6.83 *(2.81)* |
| DASS-21 Depression | |
| Total score (baseline) | 9.96 *(11.07)* |
| Total score (6 months) | 9.50 *(11.87)* |

*Table 1.* Sample characteristics (n=46); PTSD Checklist for *DSM-5* (PCL-5; Weathers et al., 2013); Childhood Trauma Questionnaire (CTQ; Bernstein & Fink, 1997); Depression, Anxiety and Stress Subscales (DASS-21; Osman et al., 2012).

*Study procedures*

All study sessions took place at the Medical College of Wisconsin. At both the acute (3 days post-injury on average; Time 1) visit and 6-month follow-up (Time 2) visit, participants underwent blood draws and completed a battery of self-report questionnaires. The Institutional Review Board (IRB) at the Medical College of Wisconsin approved all study procedures and participants were monetarily compensated for their time.

*Endocannabinoid quantification*

For the endocannabinoid assay, whole blood was extracted by trained phlebotomists at both time points. Blood was collected into 2–10 ml sterile tubes and allowed to clot at room temperature for 30–60 minutes. Samples were centrifuged using the Beckman Counter Allegra 6R swinging bucket centrifuge, at 4400 RPM and temperature of 4 ℃ for 20 minutes. Serum was pipetted via plastic disposable 5 ml pipettes at 1.0 aliquots into plastic o-ringed cryovials and immediately frozen at –80 ℃. 2-AG and AEA in the serum were determined in lipid extracts from serum using stable isotope dilution, liquid chromatography-mass spectrometry following previously published methods (Crombie et al., 2018).

*Childhood Maltreatment*

Retrospective self-reported exposure to childhood maltreatment was measured using the Childhood Trauma Questionnaire (CTQ; Bernstein & Fink, 1997). The CTQ consists of 28 items arranged into five clinical scales: emotional abuse, physical abuse, sexual abuse, emotional neglect and physical neglect. Responses are rated in terms of frequency of experiences using a 5-point Likert-type scale, with 1 = “never true” and 5 = “very often true.” The CTQ demonstrates good test-retest reliability and discriminant validity from measures of verbal intelligence and

social desirability (Bernstein et al., 1994). For the current study, CTQ (completed at 6-month visit) summed scores were calculated separately for threat experiences (emotional, physical and sexual abuse) and deprivation experiences (emotional and physical neglect).

*PTSD Symptoms*

Self-reported PTSD symptoms were measured at both time points using the PTSD Checklist for *DSM-5* (PCL-5; Weathers et al., 2013). The PCL-5 is a 20-item questionnaire corresponding to the *DSM-5* symptom criteria for PTSD. Items are rated using a 5-point Likert-type scale, with 0 = "not at all", 1 = "a little bit", 2 = "moderately", 3 = "quite a bit", and 4 = "extremely". For the current study, a total symptom severity score (range 0–80) was obtained by summing the scores for each of the 20 items.

*Depressive Symptoms*

Self-reported depressive symptoms were measured at Time 1 and Time 2 using the short-form version of the Depression, Anxiety and Stress Scales (DASS-21; Osman et al., 2012). The DASS-21 includes a depression subscale, with items rated using a 4-point Likert-type scale ranging from 0–3. For the current study, total score will be calculated by summing the scores for each of the items in the depression subscale.

*Statistical Analyses*

The current study aims to (1) examine the relationship between childhood threat and deprivation experiences and circulating AEA and 2-AG levels following traumatic injury and (2) examine whether circulating AEA and/or 2-AG in the acute post-trauma period mediate the relationship between exposure to childhood threat or deprivation and development of PTSD 6 months post-trauma.

Preliminary bivariate correlations will be conducted in order to examine the associations between threat and deprivation experiences, circulating endocannabinoids at both timepoints, and PTSD symptoms.

For Aim 1, participants' total score on the CTQ (calculated separately for threat and deprivation experiences) will be correlated with circulating endocannabinoids at both timepoints. Correction for multiple comparisons will be completed using Benjamini- Hochberg's adjustment where necessary (Benjamini & Hochberg, 1995). The specific variables included in the correlations will be CTQ threat total score, CTQ deprivation total score, 2-AG at Time 1, AEA at Time 1, 2-AG at Time 2, AEA at Time 2.

For Aim 2, mediation analyses will be conducted using PROCESS model 6 (Hayes et al., 2017) to assess whether circulating endocannabinoids partially mediate the relationship between childhood threat or deprivation experiences and PTSD symptoms. In one model, participants' self-reported experiences of childhood exposure to threat will be utilized as the predictor variable, 2-AG and AEA at Time 1 as mediators and total PTSD symptom severity at 6 months will be analyzed as the outcome variable. In a separate model, participants' self-reported experiences of childhood exposure to deprivation will be utilized as the predictor variable, with 2-AG and AEA (Time 1) as mediators and total PTSD symptoms severity (6 months) as the outcome variable. In each model, age, gender, and racial identity will be included as covariates. In follow-up analyses, I will control for PTSD symptoms at Time 1 and depressive symptoms at Time 2 given high concordance between development of depression and PTSD in trauma survivors (Kessler, 1995).

## Results

*Descriptive analyses.* Bivariate correlations among variables of interest and covariates are presented in Table 2.

| | CTQ Threat | CTQ Depr | PCL-5 (T1) | PCL-5 (T2) | AEA (T1) | AEA (T2) | 2-AG (T1) | 2-AG (T2) | DASS (T1) | DASS (T2) | Age |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CTQ Threat | 1 | .425** | .312* | .393** | -.139 | -.034 | -.125 | -.039 | .247 | .371* | -.179 |
| CTQ Depr | | 1 | .040 | -.014 | .073 | -.011 | -.141 | -.136 | .121 | .139 | -.127 |
| PCL-5 (T1) | | | 1 | .696** | .011 | -.063 | .130 | -.073 | .835** | .586** | -.307* |
| PCL-5 (T2) | | | | 1 | .014 | -.188 | .133 | .018 | .620** | .722** | -.193 |
| AEA (T1) | | | | | 1 | .399** | -.108 | .104 | -.057 | .043 | -.225 |
| AEA (T2) | | | | | | 1 | -.200 | .297* | .049 | -.031 | -.048 |
| 2-AG (T1) | | | | | | | 1 | .159 | .291* | .249 | .383** |
| 2-AG (T2) | | | | | | | | 1 | -.188 | -.115 | .293* |
| DASS (T1) | | | | | | | | | 1 | .646** | -.248 |
| DASS (T2) | | | | | | | | | | 1 | -.195 |
| Age | | | | | | | | | | | 1 |

*Table 2.* Pearson correlations between variables of interest and continuous covariates.
*$p < .05$. **$p < .01$.

Overall, greater exposure to threat was associated with increased PTSD symptom severity at baseline and 6 months post-trauma ($r$=.312, $p$=.035; $r$=.393, $p$=.007, respectively). Exposure to deprivation was not significantly associated with PTSD symptom severity at either timepoint. These correlations are presented in Figure 1. Neither exposure to threat nor deprivation was significantly associated with circulating AEA or 2-AG levels at either timepoint. Circulating AEA and 2-AG at either timepoint were not significantly correlated with PTSD symptom severity at either timepoint. Circulating AEA levels at baseline were significantly positively correlated with levels at 6 months ($r$=.399, $p$=.006) and circulating 2-AG levels at 6 months were significantly positively correlated with AEA levels at the same timepoint ($r$=.297, $p$=.045). Of note, PTSD symptom severity at baseline and 6 months post-trauma were highly positively correlated ($r$=.696, $p$<.001). Exposure to threat and deprivation experiences were also significantly positively correlated ($r$=.425, $p$=.003). Results of partial correlation analyses demonstrate that when controlling for exposure to deprivation, exposure to threat is significantly positively correlated with PTSD symptom severity at 6 months, but not baseline ($r$=.405, $p$=.006; $r$=.262, $p$=.083, respectively). Exposure to deprivation was not significantly associated with PTSD symptom severity at either timepoint when controlling for exposure to threat ($r$=-.002, $p$=.988 [baseline]; $r$=-.166, $p$=.277 [6 months]).

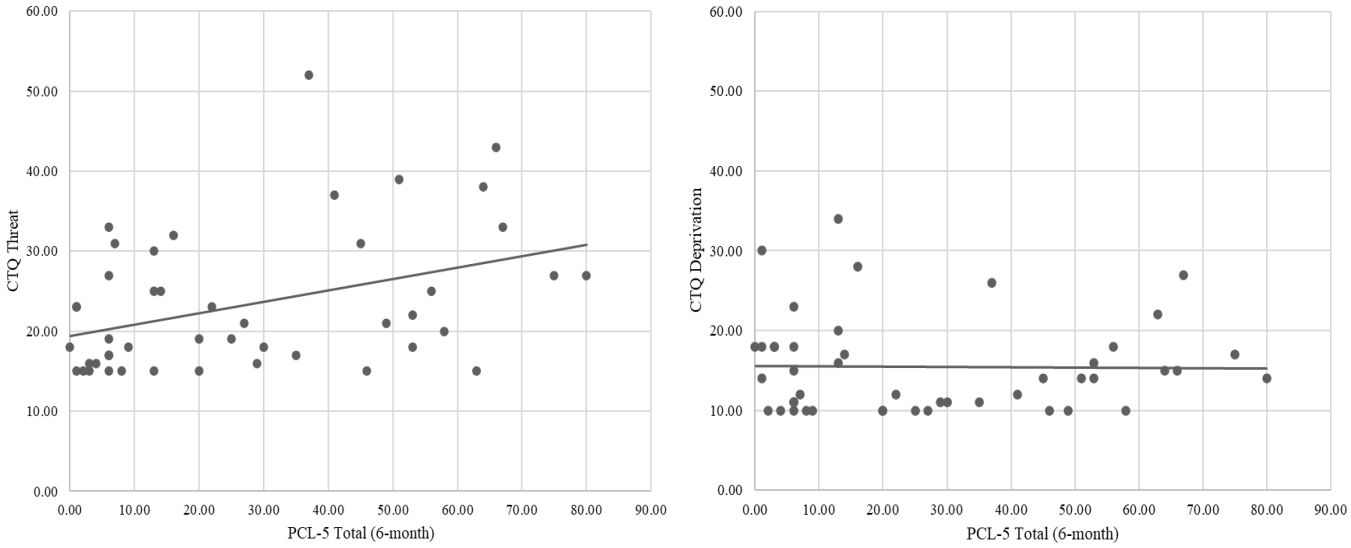


*Figure 1*. Left: Pearson correlation between CTQ Threat total score and PCL-5 total score at 6 months post-trauma ($r$=.393, $p$=.007). Right: Pearson correlation between CTQ Deprivation total score and PCL-5 total score at 6 months post-trauma ($r$=-.014, $p$=.924).

There were also significant correlations between variables of interest and covariates. Participant age at time of injury was significantly positively correlated with circulating 2-AG levels at baseline and 6-months ($r$=.383, $p$=.009; $r$=.293, $p$=.048, respectively) and negatively associated with PTSD symptom severity at baseline ($r$=-.307, $p$=.038). Results of independent samples *t*-tests revealed significant gender differences, such that participants identifying as male showed higher circulating AEA levels at both time points compared to those identifying as female ($p$=.01 [baseline]; $p$=.002 [6 months]; equal variances not assumed).

*Childhood threat and deprivation experiences and endocannabinoid tone in adulthood.* One of the primary goals of the present study was to examine whether circulating endocannabinoids (2-AG and AEA, specifically) are associated with participants' self-reported exposure to childhood threat and deprivation experiences. As illustrated in Table 2, no significant associations between total CTQ threat experiences and circulating 2-AG or AEA

levels were observed at either timepoint. Circulating endocannabinoids also did not show significant associations with total CTQ deprivation experiences.

*Mediational pathways to adult PTSD following traumatic injury.* PROCESS mediational model 6 was used to separately examine the direct effects of childhood threat and deprivation on PTSD symptom severity 6 months post-traumatic injury and the indirect effect(s) of childhood threat and deprivation on PTSD symptom severity through circulating endocannabinoid levels acutely post-trauma. In model 1, I tested whether the association between childhood threat exposure and PTSD symptom severity was partially explained by circulating endocannabinoid levels acutely post-trauma. In this model, childhood threat exposure was the predictor variable, Time 1 2-AG and AEA were mediators, and PTSD symptom severity at Time 2 was the outcome variable. Six main paths were estimated: path $a_1$, the association between childhood threat (the predictor) and 2-AG levels at Time 1 (mediator 1); path $a_2$, the association between childhood threat and AEA levels at Time 1 (mediator 2); path $b_1$, the association between 2-AG levels at Time 1 (mediator 1) and PTSD symptom severity at Time 2 (the outcome); path $b_2$, the association between AEA levels at Time 1 (mediator 2) and PTSD symptom severity at Time 2 (the outcome); path c, the total effect of childhood threat (the predictor) on PTSD symptom severity (the outcome), not adjusting for either mediator; and path c′, the association between childhood threat and PTSD symptom severity, adjusted for circulating 2-AG and AEA levels. I also tested the statistical significance of the indirect effect of childhood threat on PTSD symptom severity via bootstrapped confidence intervals. The indirect effect was considered statistically significant if the 95% confidence interval (CI) for a parameter estimate did not include a value of zero. I included age, gender and racial identity as covariates in the model.

Results for model 1 are shown in Table 3 and Figure 2. Paths c and $b_1$ were statistically significant. Paths $a_1$ and $a_2$, path $d_{21}$ and path $b_2$ were all nonsignificant. The direct effect of childhood exposure to threat on PTSD symptom severity (path c′) did not remain statistically significant when the mediational pathway was estimated. The indirect effects of childhood threat on PTSD through 2-AG, through AEA and through 2-AG and AEA in serial were all nonsignificant.

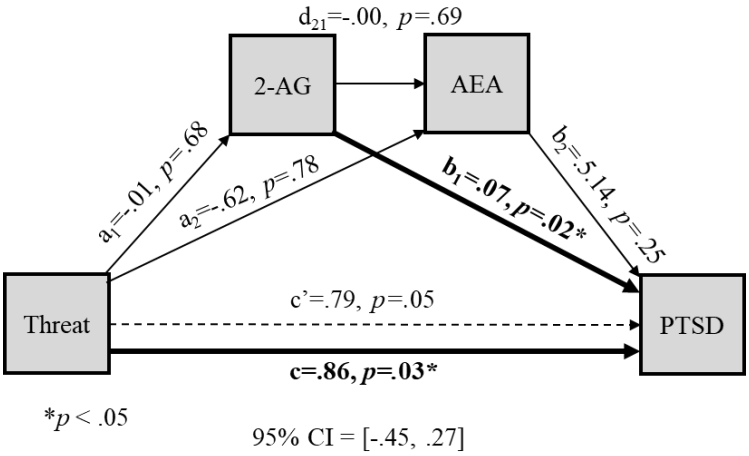


**Figure 2.** Mediational model for PTSD symptom severity with threat as predictor. The model controls for age, gender and racial identity. Dashed line denotes an effect accounting for both mediators.

| | Model 1 | | | |
|---|---|---|---|---|
| | *B* | *SE* | *t* | *P* |
| **PTSD** | | | | |
| Threat | .86 | .37 | 2.45 | **.03*** |
| 2-AG | .97 | .03 | 2.45 | **.02*** |
| AEA | 5.14 | 4.45 | 1.16 | .25 |
| Age | -.14 | .22 | -.66 | .51 |
| Gender | 10.46 | 7.43 | 1.40 | .17 |
| Race | -6.80 | 2.92 | -2.33 | **.03*** |

**Table 3.** Regression results for exposure to threat.
*$p < .05$

To further test the relationships between childhood threat exposure, circulating endocannabinoids acutely post-trauma ang PTSD symptoms severity 6-months post-trauma, I ran the same mediational model described above, with the addition of total score on the depression subscale of the DASS-21 (6-month) and PCL-5 total score (baseline) as covariates. With these covariates entered into the model, all paths were nonsignificant, including paths $b_1$ and c ($b$=.03, $p$=.34; $b$=.54, $p$=.10, respectively). PTSD symptom severity at baseline and depressive symptoms at 6 months were significantly associated with PTSD symptoms at 6 months ($b$=.42, $p$=.009; $b$=.88, $p$=.001, respectively).

Next, I ran the same analyses with childhood deprivation experiences as the predictor. As in model 1, I initially only included age, gender and racial identity as covariates in the model. Results for model 2 are shown in Table 4 and Figure 3. All direct and indirect paths were nonsignificant with the exception of path $b_1$ ($b$=.06, $p$=.03). When depressive symptoms at 6

months and PTSD symptom severity at baseline were entered as covariates, path $b_1$ was no longer significant ($b$=.00, $p$=.87). PTSD symptom severity at baseline and depressive symptoms at 6 months were significantly associated with PTSD symptoms at 6 months ($b$=.44, $p$= .007; $b$=.95, $p$=.0003, respectively).

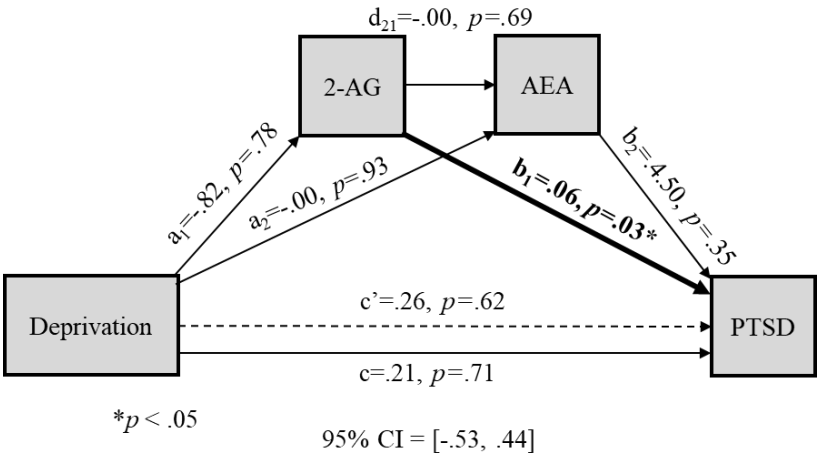


**Figure 3.** Mediational model for PTSD symptom severity with deprivation as predictor. The model controls for age, gender and racial identity. Dashed line denotes an effect accounting for both mediators.

| | Model 2 | | | |
|---|---|---|---|---|
| | *B* | *SE* | *T* | *P* |
| **PTSD** | | | | |
| Deprivation | .26 | .52 | .50 | .62 |
| 2-AG | .06 | .03 | 2.22 | **.03*** |
| AEA | 4.50 | 4.71 | .95 | .35 |
| Age | -.19 | .23 | -.83 | .41 |
| Gender | 15.44 | 7.62 | 2.03 | **.05*** |
| Race | -7.08 | 3.14 | -2.25 | **.03*** |

**Table 4.** Regression results for exposure to deprivation
*$p$ < .05

## Discussion

The overall purpose of this study was to explore and describe associations between exposure to threat and deprivation experiences in childhood, circulating endocannabinoid levels, and PTSD symptoms following a traumatic injury in adulthood. Findings from the current study add to what is known about exposure to childhood maltreatment and development of PTSD symptoms following traumatic injury in adulthood. Replicating previous findings, results show exposure to threat (in the form of physical, emotional, and/or sexual abuse), but not deprivation (emotional and/or physical neglect), in childhood is predictive of chronic PTSD symptoms following a subsequent unrelated trauma in adulthood. The current study failed to replicate a small number of studies that have demonstrated a relationship between childhood maltreatment and circulating endocannabinoid levels in adulthood. While a mediational model including childhood threat experiences as predictor, AEA and 2-AG concentrations acutely post-trauma as mediators, and PTSD symptom severity 6-months post-traumatic injury as the outcome variable was significant overall ($p$=.003), only the direct paths for 2-AG levels to PTSD symptoms and childhood threat to PTSD symptoms were significant. These findings will be discussed within the context of the existing literature and important limitations of the current study.

The present study explored the relationship between childhood exposure to threat or deprivation experiences and circulating endocannabinoids in adulthood, both in the acute aftermath of traumatic injury and 6-months post-injury. Despite my expectations, self-reported childhood exposure to threat, and separately deprivation, experiences were not significantly associated with circulating endocannabinoids at either timepoint. Due to limited sample size, the current study may not have been adequately powered to detect an effect. Thus, these results must be interpreted carefully. Aside from limitations related to sample size, there are several possible

explanations for this lack of association. First, few studies to date have investigated the effect of childhood adversity on circulating endocannabinoid levels in humans. Previous studies utilized a cumulative risk approach and did not draw a distinction between threat-related and deprivation-related childhood maltreatment or adversity. Using a cumulative risk approach, it is not possible to establish specificity in outcomes related to two distinct dimensions of childhood maltreatment (i.e., threat and deprivation), a limitation which informed the methodological approach of the current study.

Second, findings from the small number of studies on the relationship between childhood adversity and endocannabinoid concentrations are mixed and could support either increased or decreased endocannabinoid tone in individuals with a history of childhood adversity (Appiah-Kusi et al., 2020; Croissant et al., 2020; Koenig, Gao, et al., 2018; Koenig et al., 2018). It is known that differences in sampling methods across studies (type of assay collection, proximity to trauma) may contribute to disparate findings in studies utilizing peripheral measures of endocannabinoid signaling (deRoon-Cassini et al., 2020, 2022; Hillard, 2018). It is possible that, in addition to challenges posed by sampling methods and interpretation of peripheral measures of ECSS function, the relationship between childhood adversity and endocannabinoid tone is complex and difficult to capture. A potential explanation for the lack of relationship between either deprivation- or threat-related childhood experiences and circulating endocannabinoid levels in the current study could be that the relationship between endocannabinoid concentrations and childhood threat or deprivation exposure is nonlinear. For example, while some levels of exposure may result in sensitization of the ECSS response to stressors, greater frequency or severity of exposures may result in desensitization and downregulation of the ECSS response.

Third, no studies to date on the relationship between childhood maltreatment and circulating endocannabinoid concentrations have interrogated this relationship in the acute aftermath of a traumatic injury. It is possible that the ECSS response mounted in the acute aftermath of traumatic injury is so robust that it obscures individual differences in endocannabinoid tone due to early-life adversity. While it is also possible that the results of the current study may indicate that there is no true association between childhood threat or deprivation experiences and endocannabinoid tone, there is extensive evidence that exposure to threat or deprivation in childhood results in lasting alterations to neural systems (McGowan, 2013; Mehta & Binder, 2012; Shea et al., 2005; Tarullo & Gunnar, 2006). Specifically, childhood threat experiences are implicated in enduring dysregulation of the HPA axis stress response, which the ECSS is known to modulate (Hillard, 2015; McLaughlin et al., 2014; Micale & Drago, 2018). Thus, it is unlikely that childhood threat experiences do not exert influence on the ECSS during this crucial neurodevelopmental period and into adulthood. Further investigation is needed to characterize the relationship between childhood exposure to threat and deprivation and endocannabinoid tone in adulthood, particularly in the acute aftermath of a traumatic stressor.

Results of preliminary correlational analyses revealed a significant positive association between childhood threat experiences and PTSD symptom severity 6 months post-traumatic injury. This effect remained significant even when controlling for childhood deprivation experiences, which were highly correlated with childhood threat experiences. Alternatively, childhood deprivation experiences were not significantly associated with PTSD symptom severity at 6-month follow-up, even when controlling for childhood threat exposure. This is consistent with results of recent studies utilizing the DMAP model, which suggest that childhood

threat experiences, and not deprivation experiences, confer risk for fear dysregulation and trauma-related psychopathology (McLaughlin et al., 2014; Miller et al., 2018). Unexpectedly, correlational analyses indicated no significant association between circulating AEA or 2-AG levels and PTSD symptom severity, both in the acute aftermath of traumatic injury and at 6-month follow-up. This is inconsistent with a number of preclinical and clinical studies that have drawn a link between ECSS function and non-remitting PTSD, although the directionality of this association has been mixed (Berardi et al., 2016; Trezza & Campolongo, 2013; Wilker et al., 2016). Interestingly, a recent investigation by deRoon-Cassini et al. (2022), which was the first to test the association between endocannabinoid concentrations acutely post-trauma and development of non-remitting PTSD, did not find an association between concurrent measures of endocannabinoid concentrations and PTSD at 6-8 months post-trauma (deRoon-Cassini et al., 2022).

The current study tested models examining whether circulating 2-AG or AEA levels acutely post-trauma mediate the relationship between childhood threat or deprivation experiences and chronic PTSD symptoms. Consistent with prior literature (Greene et al., 2021; McLaughlin & Sheridan, 2016; Miller et al., 2018), findings indicated that childhood threat, and not deprivation, experiences positively predict PTSD symptom severity 6 months post-traumatic injury. Additionally, circulating 2-AG, and not AEA, levels acutely post-traumatic injury positively predicted PTSD symptom severity at 6 months. Notably, all indirect effects were nonsignificant, and thus 2-AG nor AEA were not shown to be mediators of the relationship between childhood threat exposure and PTSD symptoms. However, it is notable that when accounting for 2-AG and AEA in the model (path c′), the effect of childhood threat exposure on PTSD symptom severity was no longer statistically significant. It is possible that, due to limited

sample size, the current study was underpowered to detect a mediation effect that actually exists. The finding of a significant effect of acute 2-AG concentrations on PTSD symptoms is consistent with a previous finding of acute 2-AG concentrations predicting PTSD symptoms at 6 months post-trauma that trended significant (deRoon-Cassini et al., 2022). The aforementioned study reported that the relationship between acute 2-AG concentrations and PTSD symptoms at follow-up was more robust and reached significance when only participants from racially/ethnically-minoritized groups were considered. Regarding this finding, the authors cited previous findings that race-based stress represents a chronic stressor and chronic stress and high allostatic load are associated with a persistently activated ECSS (deRoon-Cassini et al., 2022; Hauer et al., 2013). Notably, the sample of the current study was largely comprised of participants from racially/ethnically minoritized groups. However, the current study failed to replicate a finding from the same study in which acute AEA concentrations predict chronic PTSD symptoms. Notably, the authors reported that this finding was more robust in women than men and sample of the current study only included a small number of women (deRoon-Cassini et al., 2022).

While the direct path between childhood threat-related experiences to PTSD was significant, the current results did not support that this was mediated by 2-AG or AEA concentrations acutely post-traumatic injury. This was unexpected, and it is possible that methodological factors made it difficult to tease apart complex interrelationships among the variables of interest. First, consistency of results across clinical studies are likely impacted by the complex nature of traumatic exposure (i.e., timing, severity, chronicity) and measurement differences (i.e., type and timing of eCB measurement, proximity to trauma). Additionally, the current study used a dimensional PTSD total symptom severity score, while some others have

utilized PTSD clinical diagnosis or have considered individual PTSD symptom clusters separately rather than combined into a total score.

On the other hand, it is possible that the results of the current study accurately find no relationship between ECSS response in the acute aftermath of traumatic exposure and PTSD symptoms. While a recent study identified individual differences in serum endocannabinoid levels acutely post-trauma as a potential marker of risk for development of PTSD (deRoon-Cassini et al., 2022), the majority of previous studies on the relationship between endocannabinoid levels and PTSD symptoms characterize endocannabinoid levels in individuals with established PTSD symptoms or diagnosis, at a distal timepoint to the index trauma. Thus, previous findings may be more reflective of alterations to the ECSS resulting from PTSD symptomatology rather than alterations to the ECSS pre-dating traumatic exposure and conferring risk for PTSD. Alternatively, recent traumatic injury has been known to result in rapid and marked changes in endocannabinoid signaling, which may have made it difficult to disentangle the effects of a more distal stressor (i.e., childhood maltreatment) and the acute influence of traumatic injury on the ECSS (deRoon-Cassini et al., 2020, 2022). While it is possible that limitations of the current study resulted in nonsignificant findings for the mediational model, it is also possible that the relationship between childhood threat experiences and chronic PTSD symptoms is not mediated by endocannabinoid signaling acutely post-trauma. Further exploration of this question with a larger and more demographically-balanced sample is warranted and would benefit from characterization of endocannabinoid tone in the pre-trauma, acutely post-trauma and long-term follow-up periods.

While the primary aims of the current study were to test relationships amongst childhood trauma and deprivation experiences, endocannabinoid concentrations and non-remitting PTSD

symptoms, additional preliminary correlational analyses were used to test for associations between variables of interest and covariates to be included in mediational tests. PTSD symptom severity at baseline and 6 months was highly positively correlated, as were AEA concentrations at baseline and 6 months. Circulating 2-AG levels at baseline and 6-months were not significantly correlated. Circulating 2-AG and AEA levels were not significantly correlated at baseline but were positively significantly correlated at 6-month follow-up. This is likely due to highly elevated 2-AG concentrations acutely post-traumatic injury, which has been found previously (deRoon-Cassini et al., 2022). Participants' total score on the depression subscale of the DASS-21 was highly correlated from baseline to 6-month follow-up, and depressive symptoms were highly correlated with PTSD symptom severity at both timepoints. This is consistent with well-established symptom overlap and high comorbidity between depression and PTSD (Kessler et al., 2018). Age at time of traumatic injury was significantly positively associated with 2-AG levels at both timepoints, consistent with previous findings (deRoon-Cassini et al., 2022). It is notable that when PTSD symptom severity at baseline and depression symptoms at 6-months were added as covariates in the mediational models, all direct paths between variables of interest were no longer significant. This could be expected given that both PTSD symptoms at baseline and depressive symptom at 6-month follow-up were highly correlated with PTSD symptoms at 6-months (the outcome variable). While PTSD symptom severity acutely post-trauma may be a more robust predictor of non-remitting PTSD symptoms overall, there is potential clinical utility in seeking to better understand the underlying mechanisms by which childhood threat and circulating 2-AG levels confer risk for PTSD.

The findings of the current study need to be considered within the context of notable limitations, especially as the results are contradictory to some previous preclinical and clinical

work. First, the current study had a relatively small sample size and may have been underpowered to detect true effects. Additionally, the sample was demographically skewed, such that the majority of participants identified as male (72%; *N*=13 female, *N*=33 male). This precluded investigation of sex differences in endocannabinoid concentrations, which have been established in previous work (Bassir Nia et al., 2019; deRoon-Cassini et al., 2022). It is also possible that the strength of the relationship between endocannabinoid concentrations and PTSD symptoms differs in male and female participants, as has been found previously (deRoon-Cassini et al., 2022). However, due to the relatively small number of female participants in the current study, this could not be investigated. Further, the majority of participants in the current sample identified as Black or African-American (76%). Investigation of racially-based differences or within-group analyses stratified by racial identity for the variables of interest was outside of the scope of this study, and was deemed inappropriate based on small sample size within each group.

Second, the current study utilized circulating endocannabinoid levels via blood assays to characterize ECSS function which has important implications for validity. Importantly, circulating endocannabinoid levels include those arising from peripheral tissues and blood cells in addition to those originating in the central nervous system (CNS). Thus, circulating endocannabinoid levels reflect endocannabinoid tone throughout the body rather than CNS endocannabinoid signaling specifically. However, several preclinical and clinical studies suggest that CNS endocannabinoid tone can influence circulating concentrations, with data showing significant associations between blood concentrations of 2-AG and AEA and CNS FAAH activity and function (Hillard, 2018). In the current study, mean circulating 2-AG concentrations at hospitalization were much higher compared to those taken at 6-month follow-up, and those typically seen in healthy controls. This was consistent with findings from another recent study in

which 2-AG concentrations were interrogated acutely post-traumatic injury (deRoon-Cassini et al., 2022). AEA concentrations were not abnormally high at either timepoint and were only correlated with 2-AG concentrations at 6-month follow-up and not acutely post-traumatic injury. This is consistent with findings that 2-AG and AEA are synthesized via different biochemical pathways and serve functionally differentiable roles in mounting of a stress response and maintaining homeostasis (deRoon-Cassini et al., 2020; Viveros et al., 2005, 2007). Additionally, due to the complexity of collecting data from acute trauma patients, time of day and food intake prior to blood draw was not standardized. These factors, in addition to time since traumatic injury, have been shown to influence circulating endocannabinoid concentrations and thus may have influenced endocannabinoid levels in the current study (Hillard, 2018).

Third, this study utilized self-report measures for variables of interest, including PTSD symptom severity and childhood maltreatment experiences. The PCL-5, a self-reported measure of PTSD symptoms, was utilized rather than a clinical interview to ascertain PTSD symptoms or diagnosis and may have been vulnerable to under- or over-reporting of subjective symptoms. The CTQ, which I utilized to quantify participants' exposure to childhood threat- and deprivation-related experiences, is a retrospective self-report measure which can bias reporting. Additionally, the CTQ does not provide data regarding timing and severity of trauma, only approximate frequency in terms of "how often" an event occurred. The age at which trauma occurs and the severity of traumatic experiences have been shown to be relevant to their impact on health outcomes (Anda et al., 2006; Ehlert, 2013; Mello et al., 2009.; Pratchett & Yehuda, 2011).

Overall, findings of the current study replicate previous findings that childhood threat, and not deprivation, experiences predict risk for development of PTSD symptoms for an unrelated traumatic experience in adulthood. Additionally, the current findings support recent

work demonstrating that the ECSS is stress-responsive and may play a role in an individual's risk or resilience for chronic PTSD symptomatology. While the presence of a mediation of the relationship between childhood threat experiences and chronic PTSD symptoms via acute endocannabinoid concentrations was not supported by the current study, future larger studies are needed to better understand the interrelationships between these variables. Further work is needed to continue to disentangle how the endocannabinoid system is influenced by early-life traumatic experiences and how it relates to risk for development for PTSD following trauma. This may serve to refine our understanding of how interventions targeting the ECSS can be the most effective in intervention and prevention of chronic PTSD.

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