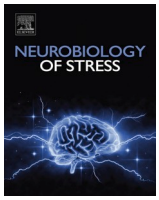
Neurobiology of Stress 30 (2024) 100634

Contents lists available at ScienceDirect

Neurobiology of Stress

journal homepage: www.elsevier.com/locate/ynstr



Predator odor stress reactivity, alcohol drinking and the endocannabinoid system

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ARTICLE INFO

Handling Editor: Rita Valentino

ABSTRACT

Post-traumatic stress disorder (PTSD) and alcohol use disorder (AUD) are highly comorbid and individual differences in response to stress suggest resilient and susceptible populations. Using animal models to target neurobiological mechanisms associated with individual variability in stress coping responses and the relationship with subsequent increases in alcohol consumption has important implications for the field of traumatic stress and alcohol disorders. The current review discusses the unique advantages of utilizing predator odor stressor exposure models, specifically using 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) on better understanding PTSD pathophysiology and neurobiological mechanisms associated with stress reactivity and subsequent increases in alcohol drinking. Furthermore, there has been increasing interest regarding the role of the endocannabinoid system in modulating behavioral responses to stress with an emphasis on stress coping and individual differences in stress-susceptibility. Therefore, the current review focuses on the topic of endocannabinoid modulation of stress reactive behaviors during and after exposure to a predator odor stressor, with implications on modulating distinctly different behavioral coping strategies.

1. Introduction

Posttraumatic Stress Disorder (PTSD) is a trauma-related disorder that develops when an individual directly experiences or witnesses a traumatic event(s). Diagnostic criteria includes intrusive distressing memories, persistent avoidance, negative alterations in cognition and mood, arousal and reactivity (APA, 2013). The emergence of symptom profiles of PTSD may occur immediately following or long after trauma exposure. Approximately 6% of the U.S. population will develop PTSD in their lifetime, while women are twice as likely to develop PTSD than men, with about 8% of women and 4% of men having PTSD at some point in their life (Goldstein et al., 2016). Interestingly, some individuals may develop PTSD symptomology, while others who go through a traumatic event may never develop PTSD. Such differences in developing PTSD suggest resilient and susceptible populations, which could be associated, in part, with specific types of behavioral responses to trauma (Center for Substance Abuse, 2014). For example, the engagement in different coping styles such as avoidance versus emotionally expressive or action-oriented versus reflective. These strategies are subjective to the individual - meaning different coping strategies are beneficial to different individuals (Center for Substance Abuse, 2014). However, there are maladaptive coping strategies to traumatic stress, such as alcohol consumption (Guinle and Sinha, 2020; Tripp et al., 2020) and engagement in compulsive and impulsive behaviors (Weiss et al., 2012), which can lead to greater psychopathology and development of comorbid disorders.

It is well known that PTSD is highly comorbid with alcohol use disorder (AUD) (Kessler et al., 1997; Sonne et al., 2003), with some individuals reporting consuming alcohol to reduce tension or stress associated with the symptoms of PTSD (i.e. self-medicating) (Lehavot et al., 2014). In addition to sex differences in the development of PTSD, women with alcohol dependence are more likely to meet the criteria for PTSD compared to men (Kessler et al., 1997). To this end, various animal models of traumatic stress have been developed which allow for the examination of individual and sex differences in comorbid AUD and PTSD, and the underlying neurobiological mechanisms.

Animal models have become increasingly important in stress research to examine behaviors that can inform our understanding of clinical PTSD-like symptoms. There are many different types of animal models of traumatic stress including stress-enhanced fear learning,

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https://doi.org/10.1016/j.ynstr.2024.100634
Received 10 January 2024; Received in revised form 26 March 2024; Accepted 30 March 2024
Available online 4 April 2024

conditioned fear models that utilize shock stressors, single prolonged stress and social defeat stress (for reviews see (Verbitsky et al., 2020; Daskalakis et al., 2013; Deslauriers et al., 2018; Whitaker et al., 2014; Cohen et al., 2012)). Other procedures such as exposure to predator odor stressors are also useful to study neural mechanisms underlying stress, as they have ethological relevance and are relatively non-invasive to rodents. Furthermore, while conditioned fear models are advantageous in studying freezing behavior (i.e. passive coping) in response to shock stressors, a unique advantage of predator odor stressor exposure, is providing rodents opportunities to engage in a multitude of behavioral coping strategies during stress. This is important because we can examine how engagement in different types of behavioral responses to stress are associated with lasting consequences to stress such as alcohol drinking. This stressor type will be the focus of this review.

Exposure to a predator odor stressor has been shown to be a valid and reliable animal model to examine symptom profiles of PTSD. For example, predator odor models can induce PTSD phenotypes including hyperarousal (Blount et al., 2023; Schwendt et al., 2018), avoidance behavior (Edwards et al., 2013; Weera et al., 2020; Albrechet-Souza et al., 2020; Albrechet-Souza and Gilpin, 2019; Brodnik et al., 2017, 2020), contextual stress memory (Ornelas et al., 2021a, 2021b; Tyler et al., 2020) and increases in plasma corticosterone levels (Ornelas et al., 2021a; Alavi et al., 2022; Finn et al., 2018; Cozzoli et al., 2014), indicative of changes in hypothalamic-pituitary-adrenal (HPA) axis activity. The HPA axis is the primary neuroendocrine system that regulates physiological and behavioral responses to stress. During stress, corticotropin releasing hormone (CRH) is released from the hypothalamus and facilitates a cascade of endocrine responses (i.e. release of glucocorticoids) that ultimately leads to the release of cortisol (corticosterone in rodents) to activate a short-term stress response. Importantly, upregulation of cortisol acts as a negative feedback mechanism that is essential in regulating HPA axis and termination of stress response. Abnormalities in HPA axis function can contribute to impairments in responding to stress and the development of PTSD (for reviews on HPA axis functioning see (De Kloet et al., 2006; Jones and Moller, 2011; Dedovic et al., 2009; Chrousos and Gold, 1992)). Lastly, there is evidence of epigenetic changes following predator odor exposure (Abuaish et al., 2021; Cuarenta et al., 2021; Brass et al., 2020; St-Cyr and McGowan, 2015; St-Cyr et al., 2017, 2018). For example, predator odor exposure to a combination of coyote urine, bobcat urine and 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) in pregnant female mice can lead to male and female adult offspring engaging in increased avoidance and having elevated corticosterone levels when exposed to a predator odor challenge (St-Cyr and McGowan, 2015). Interestingly, female offspring show elevated stress-related genes including brain-derived neurotrophic factor (*BDNF)* in the hippocampus and corticotropin-releasing hormone receptor 1 (*CRHR1)* in the amygdala, but this was not observed in males. A follow-up study showed female offspring, but not males, from predator odor-exposed dams expressed elevated transcript abundance of glucocorticoid receptor (NR3C1) and FK506 binding protein 5 (*FKBP5*) (St-Cyr et al., 2017). Finally, St-Cyr et al. (2018) showed adult offspring of predator odor-exposed dams showed an increase in transcript abundance of *Crf, Oxt* (oxytocine) and *Nr3c2* (mineralocorticoid receptor) in the Paraventricular nucleus (PVN) of the hypothalamus (St-Cyr et al., 2018). Overall, predator odor exposure models are valid and reliable models as they produce a variety of PTSD phenotypes, as well as long-term ethologically-relevant behavioral and neurobiological responses.

Predator odor models have been implemented in both mice and rat (including different strains such as Wistar, Sprague-Dawley and Long-Evans) studies to examine how predator odor stressor models affect different species and strains. Different types of predator odors include both natural odors such as bobcat urine, coyote urine, cat fur/skin odor, as well as synthetically derived compounds such as TMT (Whitaker et al., 2014; Albrechet-Souza and Gilpin, 2019; Staples, 2010; Takahashi et al., 2005). Bobcat urine has been extensively used as a predator odor avoidance model of traumatic stress that can lead to increases in alcohol self-administration (Edwards et al., 2013; Weera et al., 2020; Albrechet-Souza et al., 2020), impairments in reinforcement learning (Bielawski et al., 2022) and alterations in prefrontal cortex and amygdala functioning (Edwards et al., 2013). Importantly, this predator odor model has also been used to examine sex and individual differences (for review, see (Albrechet-Souza and Gilpin, 2019)).

Another predator odor stressor that has been extensively used in mice, is exposure to dirty rat bedding (Alavi et al., 2022; Finn et al., 2018; Cozzoli et al., 2014; Devaud et al., 2020; Manjoch et al., 2016; Clark et al., 2023; Nipper et al., 2024). This model has been used to study sex differences in response to stress, increases in ethanol intake after stressor exposure and neurochemical changes induced by predator odor stress. For example, Cozzoli et al. (2014) showed that exposure to environmental stressors including restraint, tail suspension and predator odor using dirty rat bedding to male and female mice increased plasma corticosterone levels in mice as an index of HPA activity. However, plasma corticosterone levels after all three stressors were higher in females compared to male mice (Cozzoli et al., 2014). In addition, when male and female mice were exposed to dirty rat bedding (as the first stressor that preceded other stressor tests), female rats first showed a decrease in ethanol consumption during the day of stressor exposure, and then an increase in ethanol consumption one to two days post stress (indicative of a biphasic effect); however male rats only showed an increase in ethanol consumption two days post stress (Cozzoli et al., 2014). In an interestingly follow-up study, Finn et al. (2018) showed that corticosterone levels were increased after predator odor stressors in male and female mice. However, male and female mice with prior alcohol binge drinking and exposure to intermittent predator odor exposure did not show elevated corticosterone levels, suggesting a history of binge ethanol may blunt increases in corticosterone levels in response to predator odor stress (Finn et al., 2018).

Additionally, Finn et al. (2018) examined how a history of binge ethanol drinking (measured by a lickometer) alters ethanol intake (i.e. increased lick averages) after intermittent exposure to dirty rat bedding in male and female mice (Finn et al., 2018). Male mice with a history of ethanol binge drinking showed an increase in ethanol intake after the intermittent predator odor exposures. Interestingly, female mice with a history of binge ethanol drinking produced no change in ethanol intake after predator odor exposure. However, when baseline binge intake was separated into low and high baseline subgroups based on intake, female mice with low baseline intake showed increased ethanol consumption after intermittent predator odor stressors. (Finn et al., 2018). Lastly, exposure to dirty rat bedding also showed significant sex differences in neurochemical and neuroanatomical regions associated with PTSD (Finn et al., 2018; Devaud et al., 2020). Exposure to dirty rat bedding (with and without a history of binge alcohol intake) increased glucocorticoid receptor (GR) protein expression in the PFC in female, but not male mice. In the hippocampus, both male and female mice showed increased corticotropin releasing factor receptor 1 after predator odor exposure. Finally, hippocampal GR expression was increased in female mice, but not males, after a history of binge ethanol and exposure to predator odor exposure (Alavi et al., 2022). This stressor model and a history of binge drinking has also showed significant sex differences in proteins associated in steroidogenesis and synaptic plasticity levels in the PFC and hippocampus, key regions associated with PTSD (Devaud et al., 2020). Synthetic odors derived from predator urine and/or feces, are also prevalent and valuable animal models of PTSD. An advantage of synthetically derived odorants is the likelihood of consistency from batch to batch, as the natural odorants (urine, feces) can potentially be affected by factors such as sex and diet of the predator from which they are collected. Conversely, this may also be a limitation of the single-odor molecules as they may not elicit the same range of behavioral and brain responses as natural odorants (Blanchard et al., 2003; McGregor et al., 2002). TMT was originally used to study stress and emotional responses in rats as a natural fear stimulus (Vernet-Maury et al., 1984,

1992). TMT elicits species-typical stress and fear responses including freezing, immobility and avoidance behaviors and increases in corticosterone levels (Schwendt et al., 2018; Ornelas et al., 2021a, 2021b; Tyler et al., 2020; Morrow et al., 2002; Rosen, 2004; Endres et al., 2005; Endres and Fendt, 2009; Wallace and Rosen, 2000; Rosen et al., 2015; Day et al., 2004; Thomas et al., 2006; Vendruscolo et al., 2006). In addition, our lab and others have shown male and female Long-Evans rats engage in digging behavior (similar to defensive burying) during TMT exposure (Ornelas et al., 2021a, 2021b; Tyler et al., 2020; Venton et al., 2006; Hill et al., 2006), which is a typical rodent behavior and indicative of active coping. Importantly, exposure to TMT also elicits contextual stress responses, as evidenced by increased freezing behavior during re-exposure to the exposure context (Schwendt et al., 2018; Brodnik et al., 2017, 2020; Ornelas et al., 2021a, 2021b; Tyler et al., 2020, 2022). Furthermore, our lab has shown TMT exposure produces a multitude of neurobiological and behavioral responses including engagement in stress-reactive behaviors during TMT exposure, contextual-induced stress memory, increased corticosterone levels, subsequent increases in alcohol self-administration and potentiated interoceptive sensitivity to alcohol (Ornelas et al., 2021a, 2021b; Tyler et al., 2020, 2022; Makhijani et al., 2021); while other labs have shown TMT produces alcohol reinstatement (King and Becker, 2019) and anxiety-like behavior and arousal (Schwendt et al., 2018; Brodnik et al., 2017, 2020). In addition, we and others have examined TMT exposure-induced neuroadaptations. First, TMT exposure has been shown to induce an increase in *c-fos* mRNA in male Sprague-Dawley rats specifically in regions associated with olfaction and stress, anxiety and fear-related behaviors. Regions include the olfactory bulb, bed nucleus of the stria terminalis (BNST), central and medial nuclei of the amgydala several hypothalamic regions (anterior, lateral, dorsomedial, ventromedial, paraventricular (PVN), supramammillary, and dorsal premammillary nuclei), the locus coeruleus, parabrachial nucleus and the nucleus of the solitary tract (Day et al., 2004). Janitzkey et al. (2015) showed TMT-induced increases in Fos-positive cells in the CeA, PVN and BNST, all of which are important regions that regulate behavioral and endocrine stress responses (Janitzky et al., 2015). Asok et al., 2013 examined changes in immediate early genes *c-fos and* early growth response 1 *(egr-1),* as well as gene expression levels of corticotropin-releasing hormone *(crh)* and *enk* in the BNST, PVN, central nucleus of the amygdala (CeA) and medial prefrontal cortex (mPFC) after water, butyric acid and TMT exposure in male Sprague-Dawley rats. Importantly, *c-fos* and *egr-1* were elevated in the CeA, PVN and BNST, while *crh* were elevated in the CeA and PVN but none were increased in the mPFC in TMT-exposed male rats (Asok et al., 2013). Therefore, TMT induces changes in gene expression in key regions associated with regulating innate fear behavior. Tyler et al. (2020) showed short and long-term changes in gene expression in male Long-Evans rats following TMT exposure related to stress and excitatory synaptic functioning in brain regions associated with PTSD. Specifically, TMT produced an upregulation in *FKBP5* in the hypothalamus 6 h after TMT and in the dorsal hippocampus four weeks after TMT exposure, (Tyler et al., 2020). *GRM5* (encodes for mGluR5) was upregulated in the prelimbic cortex, while *CNR1* (encodes for CB1) was decreased in the ventral hippocampus four weeks after TMT. Therefore, this study showed immediate and lasting molecular changes following TMT predator odor exposure (Tyler et al., 2020). Overall, TMT exposure is a valuable model to study different symptom profiles of PTSD outlined in DSM-V diagnostic criteria including avoidance, alterations in arousal and reactivity and experiencing intrusive distressing memories of a traumatic event(s) (APA, 2013). Similar to other predator odor exposure models, TMT exposure can be used to examine individual differences in response to stress and how this individual variability may contribute, in part, to susceptible and resilient populations in developing PTSD and AUD.

2. Stress reactivity: individual differences and implications for alcohol drinking

As previously mentioned not all individuals who experience a traumatic event develop PTSD and individuals cope with trauma in many different ways. Therefore, understanding the neurobiological mechanisms associated with individual differences in coping behaviors during and after trauma is important and may provide insight in understanding susceptible versus resilient factors associated with the development of PTSD and comorbid disorders. Animal models of predator odor stress, such as bobcat urine can be used to examine contextual avoidance to an odor-paired chamber in rats (Edwards et al., 2013; Weera et al., 2020; Albrechet-Souza et al., 2020, 2021; Albrechet-Souza and Gilpin, 2019; Templeton et al., 2023). This is important as experiencing intrusive distressing memories of traumatic event(s) is a DSM-5 diagnostic criteria for PTSD (APA, 2013), and can lead to avoidance behavior. This predator odor avoidance model of PTSD can be used to examine behavioral and biological outcomes associated with avoidance stress coping behavior. For example, male Wistar rats classified as “Avoiders” exhibit escalated and compulsive-like alcohol self-administration after exposure to bobcat urine compared to rats classified as “Non-Avoiders” (Weera et al., 2020). Avoider rats exhibit greater c-Fos-positive and corticotropin-releasing factor (CRF) immunoreactivity in the CeA, a region well-implicated in PTSD (Weera et al., 2020). Further, antagonism of CRF in the CeA reduces escalations in alcohol self-administration in Avoider rats, as well as avoidance behavior during bobcat urine exposure (Weera et al., 2020). Therefore, this model is able to capture individual differences in response to stress and brain mechanisms associated with heterogeneity in stress-induced avoidance behavior.

In mice, repeated exposure to dirty rat bedding can also be used to subgroup rats into “sensitive” and “resilient” phenotypes based on percent change in ethanol drinking before and after repeated stressor exposure (Alavi et al., 2022). Mice that show approximately 20% increase in home cage alcohol drinking after the stressor exposure compared to baseline drinking are defined as “sensitive”, while “resilient” mice show decreased or no change in drinking after the stressor (Alavi et al., 2022). This method to examine individual differences focuses on drinking behavior rather than behavioral coping strategies during the stressor such as avoidance or immobility. Importantly, this animal model of PTSD is advantageous to examine neurobiological mechanisms associated in regulating alcohol drinking after traumatic stress. For example, Clark et al. (2021) examined c-Fos activation between sensitive and resilient phenotypes in male and female mice after repeated exposure to dirty rat bedding. Male mice in the sensitive subgroup demonstrated higher c-Fos induction in the paraventricular nucleus of the hypothalamus (PVH) and piriform cortex (Pir) (Clark et al., 2023). This is an important finding as it suggests male mice with greater sensitivity (i.e. increased alcohol drinking) to repeated predator odor stress have increased HPA axis activity, suggestive of greater stressor response and potentially indicative of a maladaptive neuroendocrine response to stress (Clark et al., 2023). Furthermore, Alavi et al. (2022) showed that in the PFC, CRF receptor 1,2, binding protein and GR were higher in female mice in the sensitive subgroup, but this was not found in males. These data suggest sex differences in expression of proteins that regulate stress responses, in a key region implicated in PTSD (Alavi et al., 2022). Predator odor exposure using TMT has also been used to examine individual differences in response to stress, as well as a rat model of comorbid PTSD and substance use disorders including cocaine use disorder (Schwendt et al., 2018; Brodnik et al., 2017, 2020) and AUD (Ornelas et al., 2021a; King and Becker, 2019; Becker et al., 2023). For example, Brodnik et al. (2017) exposed male Sprague-Dawley rats to TMT then sub-grouped rats into a “susceptible” group based on time spent in open arms during elevated plus maze and avoidance behavior when re-exposed to the odor-paired context (Brodnik et al., 2017). Susceptible rats spent less time in open arms during the elevated plus maze indicative of anxiety-like behavior, showed increased avoidance

when re-exposed to the odor-paired context, increased acoustic startle response and corticosterone levels. Importantly, susceptible rats showed increased motivation to self-administer cocaine, as well as sensitization to dopaminergic effects of cocaine (Brodnik et al., 2017). Similarly, to study individual differences in traumatic stress and cocaine use disorder, Schwendt et al. (2018) exposed male Sprague-Dawley rats to TMT and sub-grouped rats into resilient or susceptible phenotypes based on anxiety-like behavior during the elevated plus maze and acoustic startle response (ASR) (Schwendt et al., 2018). Importantly, during cocaine self-administration, extinction, and cue primed reinstatement paradigms, susceptible rats showed greater cocaine lever presses during extinction learning sessions, as well as increased cue-primed reinstatement; this was not shown in resilient rats (Schwendt et al., 2018).

To study individual differences in behavioral response to TMT, Hebb et al. (2004) exposed male mice to TMT and examined TMT-induced anxiety-like behavior during light/dark test as well as mesocorticolimbic neuronal activity and encephalin (ENK) expression (Hebb et al., 2004). This study subgrouped mice into two separate methods, 1) low/high odor responders based on time spent freezing during TMT exposure and 2) low/high-anxiety groups based on total time spent in light chamber during light/dark test after TMT exposure. First, high-responding (increased TMT-induced freezing) male mice showed reduced time spent in the light chamber, indicative of anxiety-like behavior, immediately after TMT exposure. High responding male mice showed an increase in fos-related antigen (FRA) immunoreactivity (indicative of neuronal activation) and neuronal activation of ENK neurons in the nucleus accumbens shell. Next, high-anxiety male mice showed increased FRA immunoreactivity in the prelimbic cortex (PrL), infralimbic (IL) cortex and nucleus accumbens shell. Increased neuronal activation of ENK neurons was also shown in the baseolateral amygdala (BLA) and central amygdala (CeA) in high-anxiety male mice. These data are important as they suggest individual responsivity to TMT, as well as TMT-induced anxiety-like behavior, is associated with increase in neuronal activity and ENK gene regulation in mesolimbicortical regions associated with regulating motivation, reward, fear and anxiety (Hebb et al., 2004).

Our lab has shown that individual differences in stress reactivity/coping during the TMT exposure can predict subsequent alcohol drinking and can be used to examine the complex interaction between increased alcohol consumption and stress, which may help in understanding the neuroadaptations that underlie comorbid PTSD and AUD. In addition, we have examined how TMT can elicit distinct behavioral phenotypes in male and female rats during TMT exposure and how these phenotypes are associated with increases in alcohol drinking after stress (Ornelas et al., 2021a). During the TMT exposure, male and female Long-Evans rats engage in two distinctly different stress-reactive behaviors including defensive digging (i.e. active coping) and immobility behavior (i.e. passive coping) (Ornelas et al., 2021a, 2021b; Tyler et al., 2020). Using these two stress-reactive behaviors, we can quantify rats into active (i.e. high digging behavior) versus passive (i.e. high immobility behavior) coping subgroups to measure individual differences in stress responsivity. Interestingly, we find that male rats engage in greater passive coping behavior during TMT exposure while females engage in greater active coping behavior. In addition, female rats that engage in greater active coping behavior 1) show greater contextual-stress memory indicative of increased digging behavior during re-exposure to TMT-paired context and 2) increased alcohol self-administration (Ornelas et al., 2021a). However, these increases in self-administration were not shown in males (Ornelas et al., 2021a). Therefore, because we quantify behaviors during TMT exposure, we can assess how different phenotypical behavior in rats are associated with lasting consequences to stress including alcohol consumption. Interestingly, our lab has shown that exposure to TMT produces an increase in interoceptive sensitivity to alcohol in male rats (Tyler et al., 2022). In this study, male rats were trained on operant alcohol discrimination, which provided an extensive history of alcohol exposure. During TMT exposure, these ratsshowed an enhanced hyperactive response (i.e. greater digging behavior, increased distance traveled and midline crossings in exposure chamber and little avoidance or immobility behavior) in comparison to an alcohol-naïve male cohort that engaged in more passive behaviors including immobility and avoidance behavior (similar to behavioral findings in Ornelas et al. (2021)). In addition, the male rats trained on operant alcohol discrimination (i.e alcohol-experienced) and exposed to TMT showed potentiated sensitivity to the interoceptive effects of alcohol. Therefore, while Ornelas et al. (2021) showed no increases in alcohol self-administration in male rats after TMT exposure, an important distinction with Tyler et al. (2022) is the extensive history of alcohol exposure prior to TMT exposure. These data are important as they suggest a history of alcohol drinking may promote more hyperactive behaviors during TMT exposure, potentiate interoceptive sensitivity to alcohol after TMT, which may be involved in preventing escalations in alcohol drinking after stress (Tyler et al., 2022). Lastly, a critical contribution of these predator odor stress models is that they can be used to examine individual differences in the underlying neurobiological mechanisms and circuitry associated with susceptible or resilient populations.

3. Endocannabinoids: modulating stress reactivity in response to predator odor exposure

The endocannabinoid system has been implicated in modulating many aspects associated with PTSD (for review see (Hill et al., 2023; Mayo et al., 2022; Hill et al., 2018; Patel et al., 2017; Neumeister et al., 2015; Lutz et al., 2015; Gunduz-Cinar, 2021; Bedse et al., 2020)). For example, the endocannabinoid system can act as an endogenous protective or defense mechanism against stress (for review see (Morena et al., 2016)) by regulating stress signaling in the brain (McLaughlin et al., 2012) including the balance of inhibitory and excitatory transmission during stressor exposure and allowing for appropriate cortical control of stress responses (Fitzgerald et al., 2019; Kiritoshi et al., 2013; Rey et al., 2012). In brief, modulation of neuronal transmission through activation of cannabinoid receptor (CB1) receptor through endogenous ligands N-arachidonoyl ethanolamine (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG), appears to be primarily localized to GABAergic and glutamatergic terminals (Freund et al., 2003). AEA and 2-AG are synthesized in the post-synaptic neuron and act as retrograde transmitters to reduce neurotransmitter release probability from the presynaptic neuron (for extensive review on endocannabinoid signaling see (Freund et al., 2003; Ahn et al., 2008)). Therefore, endocannabinoid signaling can regulate both excitatory and inhibitory synaptic transmission (Rey et al., 2012), which is known to play a major role in regulating neuronal activity associated with traumatic stress (Morena et al., 2016; Freund et al., 2003). Therefore, the endocannabinoid system is an important target to consider when understanding how to modulate stress-related biological processes associated with PTSD, as well as neurobiological mechanisms associated with stress susceptibility phenotypes. Given the abundance of reviews on the role of the endocannabinoid system in PTSD, we limit the scope of this section of the review to the topic of endocannabinoid modulation of stress-reactivity during and after exposure to predator odor stress to understand how the endocannabinoid system is involved in regulating behavior in response to traumatic stress.

Preclinical work strongly suggests that the endocannabinoid system may directly modulate behavioral aspects of PTSD including fear extinction and contextual stress memory (Gunduz-Cinar et al., 2023; Huckleberry et al., 2023; Zabik et al., 2023; Crombie et al., 2022; Morena et al., 2018, 2021; Mayo et al., 2020; Balogh et al., 2019; Fidelman et al., 2018), and stress coping responses (Hill et al., 2006; McLaughlin et al., 2012, 2013, 2014; Kondev et al., 2022, 2023; Steiner et al., 2008; Gunduz-Cinar et al., 2013; Haller et al., 2013; Bluett et al., 2017; Marcus et al., 2020; Berger et al., 2018; Pavón et al., 2021; Metna-Laurent et al., 2012). During fear conditioning, deletion of CB_1

receptors in male mice prevents temporal shift from engagement in freezing behavior to active coping behavior (Metna-Laurent et al., 2012). Specifically, CB_1 deletion overall reduces engagement in freezing behavior and mice displayed very little, if any, active coping behavior. These data suggest the endocannabinoid system plays a major role in determining which coping strategy rats engage in during fearful or stressful situations (Metna-Laurent et al., 2012). As previously stated, predator odor stressors are ethologically relevant stressors that can induce innate fear and stress responses. Thus, using pharmacological manipulation approaches, the endocannabinoid system can either be augmented or suppressed to examine how alterations in this system can modulate behavioral responses to stress. Pharmacological approaches include inhibiting enzymes that metabolize 2-AG (i.e. monoacylglycerol lipase, MAGL) and AEA (i.e. fatty acid amide hydrolase, FAAH) (Blankman and Cravatt, 2013), as well as administration of transport inhibitors, increase endocannabinoid levels (see Table 1 for summary). Furthermore, pharmacological inhibition of the enzyme diacylglycerol lipase (DAGL) which converts diacylglycerol to 2-AG, can also be used to study the effects of 2-AG depletion. Hill et al. (2006) showed that stress reactive behaviors during TMT exposure could be directly modulated via pharmacological administration of an endocannabinoid uptake inhibitor, AM404 (increases anandamide levels). Male Sprague-Dawley rats treated with AM404 prior to TMT exposure, showed a significant reduction in the engagement in defensive burying behavior (Hill et al., 2006), suggesting that pharmacological augmentation of endocannabinoid activity can reduce TMT-induced behavioral responses (Hill et al., 2006). Additionally, as previously mentioned, rats engage in a variety of behavioral responses during TMT exposure and the endocannabinoid system may differentially modulate these behaviors, as they are distinctly different in response. For example, exposure to the synthetic predator odor compound 2 MT (2-methyl-2-thiazoline; similar compound to TMT) has been shown to induce innate behavioral stress responses in both male and female mice such as freezing and avoidance behavior, and produce elevations in 2-AG levels in the amygdala and decreased 2-AG levels in the prefrontal cortex (PFC) (Kondev et al., 2022). Systemic injection of JZL-184, a MAGL inhibitor (reduces 2-AG metabolism), prior to 2 MT exposure increased freezing behavior during exposure in both male and female mice, but did not alter avoidance behavior. When mice were re-exposed to the 2 MT-paired context 2–6 days later, there was a reduction in conditioned freezing behavior. This is an important finding as it suggests MAGL inhibition can differentially mediate innate freezing responses to predator odor and conditioned freezing responses (Kondev et al., 2022). Overall, these data suggest that endocannabinoid modulation of stress-reactive behaviors during predator odor exposure may be specific to the nature of the behavior (e.g., defensive burying, freezing, avoidance).

Table 1
Pharmacological Modulation of eCB System and Associated Behavioral Responses to TMT Exposure.

Behavioral Responses during TMT Exposure			
Subject	Pharmacological Agent	Pharmacological Effect	Behavioral Response
Rats	AM404 (Hill et al., 2006)	Inhibitor of AEA reuptake	↓ Defensive Digging
Mice	JZL-184 (Kondev et al., 2022)	MAGL inhibitor	↑ Freezing (2-TMT)
Lasting Behavioral Consequences to TMT Exposure			
Rats	JZL-184 (Lim et al., 2016)	MAGL inhibitor	↓ TMT-induced anxiety-like behavior (during EPM)
Rats	URB597 (Danandeh et al., 2018) URB937 (Carnevali et al., 2023) ARN14280 (Fotio et al., 2023) ARN14663 (Fotio et al., 2023)	FAAH inhibitor	↓ TMT-induced anxiety-like behavior (during EPM)

Abbreviations: Anandamide (AEA), monoacylglycerol lipase (MAGL), fatty acid amide hydrolase (FAAH). **Pharmacological agents**: AM404-increases AEA levels; JZL-184- reduces 2-AG metabolism, increases 2-AG levels; URB597, URB937, ARN14280, ARN14663- reduces AEA metabolism, increases AEA levels.

Endocannabinoid signaling has also been known to regulate long-term consequences of predator odor stressor including anxiety-like behavior (Lim et al., 2016; Ivy et al., 2020; Danandeh et al., 2018). Lim et al. (2016) showed TMT induces sustained increases in anxiety-like behavior during EPM and elevated 2-AG levels in the BLA in male Sprague-Dawley rats (Lim et al., 2016). Furthermore, rats were clustered into TMT-resistant and TMT-sensitive subgroups using anxiety-like behavior during EPM. TMT-sensitive rats showed show high levels of anxiety-like behavior and elevated 2-AG levels in the BLA. Interestingly, systemic administration of JZL-184 after TMT exposure reduced anxiety-like behavior in EPM 7 days post-TMT exposure (Lim et al., 2016), showing that MAGL inhibition can attenuate long-term behavioral consequences of TMT exposure. In addition to 2-AG, anandamide (AEA) has also been implicated in modulating behavioral responses to stress (Danandeh et al., 2018; Carnevali et al., 2023; Fotio et al., 2023). For example, male Sprague-Dawley rats systemically injected with URB597, a FAAH inhibitor, after TMT exposure showed significantly reduced anxiety-like behavior in the EPM one week later as compared to rats exposed to TMT but treated with vehicle. This reduction in TMT-induced anxiety-like behavior was correlated with inhibition of brain FAAH activity and increased brain levels of AEA (Danandeh et al., 2018). The role of endocannabinoid signaling has also been implicated in modulating acoustic startle reactivity, specifically in female Wistar rats, after exposure to bobcat urine (Albrechet-Souza et al., 2021). First, it has been previously shown that female rats exposed to bobcat urine show blunted acoustic startle response after stressor exposure, indicative of a hypoarousal response (Albrechet-Souza et al., 2020). In addition, female rats exposed to bobcat urine show blunted 2-AG levels in the BLA and elevated AEA levels in the CeA (Albrechet-Souza et al., 2021). Interestingly, intra-BLA injection of DO34 (DAGL inhibitor; leads to depleted levels of 2-AG) and intra-CeA injection of URB597 (FAAH inhibitor), enhanced acoustic startle reactivity after exposure to bobcat urine. These findings suggest differential effects of predator odor stress on endocannabinoid levels in amygdala nuclei and further suggest manipulation of the endocannabinoid signaling specifically in the amygdala, can modulate stress-reactivity in response to predator odor stress (Albrechet-Souza et al., 2021). Overall, these studies indicate both 2-AG and AEA-mediated signaling can modulate both stress-reactivity during predator odor exposure as well as long-term behavioral consequences of the exposure and add to the large body of work implicating the vital role of the endocannabinoid system in modulating stress responses.

4. Conclusion

In conclusion, predator odor exposure models are valid and reliable animal models to study behavioral and neurobiological mechanisms associated with PTSD, AUD and individual responses to stress (summarized in Fig. 1). These models can induce PTSD phenotypes and alterations in alcohol drinking after stressor exposures. Importantly, they are valuable models to capture individual differences in response to stress and brain mechanisms associated with heterogeneity in stress-induced coping behaviors. Furthermore, these models are critical in examining neurobiological mechanisms that can modulate stress reactivity, including the endocannabinoid system. Importantly, the endocannabinoid system is involved in regulating stress-reactivity specifically stress coping and individual differences in stress-

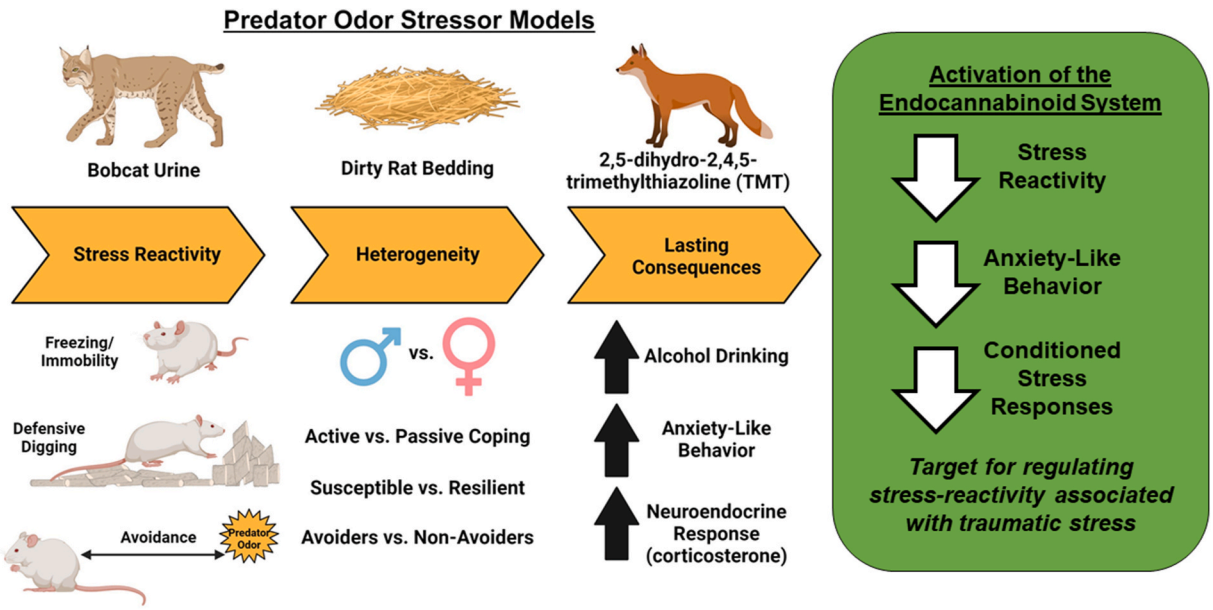

Fig. 1. Predator exposure models induce individual differences is stress reactive behaviours and produce lasting consequences on behaviour associated with PTSD symptomology. Activation of the endocannabinoid system can regulate stress-reactivity specifically stress copying individual differences in stress-susceptibility.

susceptibility. In addition, cannabinoid receptor ligands such as 2-AG and AEA have been shown to regulate anxiety-like behavior and promote stress responsivity. Overall, predator odor exposure models are highly valuable for understanding PTSD pathophysiology and can be utilized to test pharmacological treatments for PTSD, making these models advantageous for developing improved treatment and prevention strategies for PTSD.

CRediT authorship contribution statement

Laura C. Ornelas: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Joyce Besheer:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

Supported in part by AA029730 (LCO) and AA026537 (JB) and the Bowles Center for Alcohol Studies.

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