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Endocannabinoid System Alterations in Posttraumatic Stress Disorder: A Review of Developmental and Accumulative Effects of Trauma

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Abstract

The role of the endocannabinoid system in stress-related psychiatric symptoms has been investigated in many animal and human studies. Although most of these studies consistently report long-lasting effects of prolonged stress and trauma on the endocannabinoid system, the nature and direction of these changes are controversial. We reviewed the available preclinical and clinical studies investigating the endocannabinoid system alterations long after chronic stress and trauma. We propose that the effects of prolonged stress or trauma on the endocannabinoid system are different based on the developmental age of subjects at the time of experiencing the trauma and its repetitiveness and accumulative effects. The current literature consistently demonstrates decreased levels of endocannabinoid ligands and receptors if the trauma occurs in childhood, whereas decreased levels of endocannabinoid ligands and increased levels of cannabinoid receptors are reported when trauma has happened in adulthood. It is important to note that these changes are region-specific in the brain and also there are important sex differences, which are beyond the scope of this review.

Keywords

childhood trauma, endocannabinoid, PTSD, stress, trauma

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Introduction

The potential role of the endocannabinoid (eCB) system in the treatment of posttraumatic stress disorder (PTSD) has attracted increasing attention over the past decade.[1] The eCB system has an essential role in stress response, and accumulating evidence suggests long-lasting eCB system alterations in response to stress and trauma.[2] These alterations seem to have a critical role in the development and maintenance of stress-related psychopathology, as these alterations usually last long after the termination of the trauma.[3,4] Although the vast majority of the preclinical studies are consistent in existence of the eCB system alterations among individuals who were exposed to trauma,[5] it seems that there is no consensus on the nature of these changes. Interestingly, individuals with histories of trauma and PTSD are associated with a high rate of cannabis use[6,7] and PTSD is, in fact, one of the main psychiatric indicators of medical marijuana use.[8,9] Nevertheless, several studies report higher rates of anxiety and more severe PTSD symptoms in individuals diagnosed with PTSD and comorbid cannabis use.[8,10] At the same time, an increasing line of animal studies demonstrate beneficial effects of cannabinoids and eCB enhancers in improving PTSD-like symptoms[11–13] and ongoing clinical trials have been investigating these effects in humans.

Many confounding factors or differences in research methodologies may explain the inconsistencies in preclinical and clinical studies on eCB system alterations in PTSD. Here, we propose that trauma-related factors including its repetitiveness and chronicity as well as the developmental age of subjects when first exposed to

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trauma play an important role in the nature of induced eCB system changes. We summarize the current preclinical and clinical studies on the eCB system alterations in response to trauma and chronic stress and categorize them based on the trauma factors. We thus explore them in the context of early life stress, chronic repetitive unpredictable stress, and single adulthood severe trauma and will focus on the cannabinoid receptor 1 (CB1R) and eCB ligands alterations.

eCB System

The eCB system is a neuromodulatory system that is comprised of CB1R and CB2R, respectively, and two main endogenous lipid eCBs N-arachidonylethanolamine (AEA, also called anandamide) and 2-arachidonoylglycerol (2-AG). There has long been evidence that the eCB system plays a critical role in regulating the stress response.[2] The stress response acts to restore homeostasis in an organism and to promote survival in response to real and perceived threats, and it includes the activation of an autonomic response through the sympathetic nervous system in addition to a neuroendocrine response primarily driven by the hypothalamic-pituitary-adrenal (HPA) axis.[3] Evidence suggests that the eCB system plays an important role in constraining this neuroendocrine response.[14] A brief introduction to the eCB system and the current understanding of the role the eCB system plays in the stress response is presented here.

Cannabinoid receptors are differentially distributed throughout the brain and periphery. CB1Rs are primarily found within the central nervous system (with particularly high densities within the cortex, amygdala, hippocampus, and basal ganglia), while CB2Rs are thought to be predominantly peripheral and distributed heavily within tissues throughout the immune system.[15,16] More recent evidence suggests that there is in fact some expression of CB2R within mammalian brains that may play an active role in neuromodulation, but more work is needed to further characterize the potential role CB2R activity may play within central signaling networks. In this review, we will focus on the CB1R since the majority of studies on eCB signaling in trauma and stress have investigated the role and alterations of these receptors in the brain.

Cannabinoid receptors are presynaptic G protein-coupled receptors[17–19] expressed on axon terminals of glutamatergic, GABAergic, and some monoaminergic neurons.[20] Synthesis of endogenous eCB occurs "on-demand," meaning that the activation of cannabinoid receptors is coupled to endogenous eCB synthesis.[2,21] Synthesis occurs in the postsynaptic neuron, followed by retrograde signaling back onto cannabinoid receptors on the presynaptic terminal.[19,22] CB1R activation ultimately results in suppression of neurotransmitter release (primarily glutamate or GABA) within the synapse, thus possessing the ability to modulate activity at both excitatory and inhibitory synapses.[4,18,20,23] Disruption of CB1R activity is associated with an increased anxiety phenotype in animal models, while agonism of the CB1R results in behavior changes consistent with reduced anxiety.[1,24]

AEA and 2-AG are the main endogenous eCBs within the eCB system. AEA was initially discovered in 1992[25] with the discovering of 2-AG following soon after.[26] Other less studied N-acylethanolamines that may represent future therapeutic targets include N-stearoylethanolamide (SEA), N-palmitoylethanolamide (PEA), and N-oleoylethanolamide (OEA).[27,28] These compounds may have an "entourage" effect within the eCB system, not themselves acting on the CB1R but instead potentiating the effect of AEA.[28,29] While physiologically AEA and 2-AG have similar actions, they seem to serve differing functional roles within the stress-response system. Evidence suggests that AEA maintains the "tone" of neurotransmitter release in the nonthreatened steady-state,[30,31] while 2-AG represents a more "phasic" response that takes place after neuronal depolarization and mediates return to baseline, in addition to contributing to forms of synaptic plasticity.[32,33]

AEA and 2-AG are predominantly metabolized by distinct hydrolases. Fatty acid amide hydrolase (FAAH) is the major metabolizer of AEA,[34] while 2-AG is primarily degraded by monoacylglyceride lipase (MAGL).[35] These enzymes are important regulators of the eCB system and represent an active area of research into potential pharmacologic targets for various stress-related psychiatric disorders.[21,36] Evidence consistently shows that pharmacologic FAAH inhibition promotes fear extinction and reduces anxious behaviors in rodent models.[37,38] In humans, a loss of function allele resulting in reduced FAAH activity (and subsequent increased basal AEA levels) has also been shown to facilitate fear extinction and was associated with lower trait anxiety and stress reactivity scores as well as decreased amygdala threat reactivity on imaging studies.[38–40]

Strong evidence supports the role of eCB signaling in regulation of the HPA axis. The accumulated evidence suggests a model where the eCB system functions to constrain the HPA axis during nonstressed conditions, with loss of that tone during acute stress resulting in increased HPA axis activity.[30,31] This loss of "tonic" inhibition by the eCB system is thought to be mediated by a corticotropin-releasing hormone (CRH) which increases FAAH activity.[23,41] Increased FAAH activity during acute stress lowers concentrations of AEA within the basolateral amygdala, freeing the HPA axis from the constraints of the eCB system.[42,43] In addition, the eCB system participates in a delayed negative feedback response to help bring HPA axis activity back towards homeostasis.[14,23,31,32]

This negative feedback has been demonstrated at the level of the hypothalamus within the periventricular nucleus (PVN).[17,18] Glucocorticoid signaling mobilizes eCBs within the PVN which suppress glutamate release into the synapse via activation of CB1R, ultimately suppressing further excitatory drive onto CRH neurons and buffering the stress response.[31,33] Similarly, evidence suggests that the eCB system also participates in negative feedback of the HPA axis within the prefrontal cortex (PFC). In this respect, stress via glucocorticoid activity increases 2-AG content within the PFC, leading to suppression of neurotransmitter release and modification of inhibitory tone locally,[44] ultimately contributing to blunting of the stress response.

Consistent with the above findings, antagonism or deletion of CB1R activity results in a particularly robust and prolonged activation of the HPA axis by threats.[31,42,44] The buffering effect of the eCB system seems to be context specific and particularly important during episodes of acute stress; disturbed CB1R signaling may somewhat impact basal anxiety levels under nonstressed conditions, but under stressed conditions this impact is dramatically increased.[24,31,45] This concept has been observed in human studies; for example, human participants receiving a cannabinoid antagonist/inverse agonist were found to have significantly increased situational anxiety levels following a stressful event compared to those receiving placebo, but prestress anxiety levels between groups were similar.[45]

The Acute Effects of Stress on the eCB System

As recently reviewed by Morena et al., stress has complex effects on the eCB system that vary by region and time course.[5] In general, as described earlier and summarized in Figure 1, AEA is thought to represent a gatekeeper on the HPA axis during the nonstressed state. Loss of AEA tone during acute stress results in increased HPA activity, facilitating the stress response. This rapid reduction in AEA following an acute stressor has been demonstrated in the amygdala[42,43] and hippocampus[32] and is attributed to increased CRH-mediated hydrolysis by FAAH.[41] This reduction in AEA activity following acute stress is not consistently observed in PFC,[41,44] suggesting possible site-specific variations in physiological processes as described earlier. Consistent with the "tonic" and "phasic" model of AEA and 2-AG function, acute stress generally results in an increase in tissue 2-AG content.[5,44] Increased 2-AG following an acute stressor has been observed within the hypothalamus,[33] hippocampus,[32] and PFC,[44] but not within the amygdala.[42] While reductions in AEA following stress occur rapidly, elevations in 2-AG are relatively delayed, consistent with the current understanding that reduction in AEA is mediated by CRH and occurs prior to the glucocorticoid response, while increases in 2-AG following acute stress seem to be mediated by glucocorticoids.[5,17,44] More studies are needed to further understand the specific role of eCB system in different brain regions in response to acute stress.[5,14]

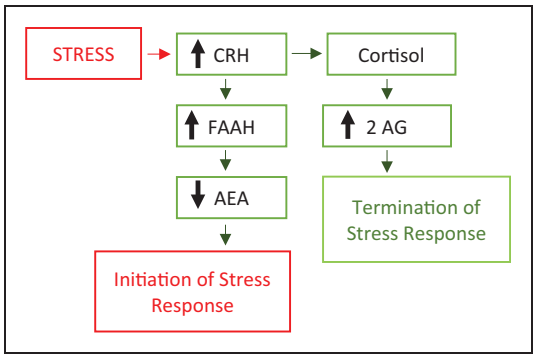

Figure 1. Role of eCB system in acute stress response. CRH: corticotropin-releasing hormone; FAAH: Fatty acid amide hydrolase; AEA: N-arachidonylethanolamine; 2-AG: 2-arachidonoylglycerol.

Long-Term Effects of Trauma on eCB System

Preclinical Studies

Early Life Stress. Childhood trauma is associated with several psychiatric sequela such as depression, anxiety, PTSD, borderline personality disorder (BPD), and psychosis. Maternal deprivation (MD) early in life has been used as a model of childhood trauma and has been shown to induce depressive-like and psychotic-like symptoms. In these studies, rodents' mother is typically removed from their cage on postnatal day (PND) 9 for 24 h. Several investigators have explored the effects of MD on the eCB system. Here, we summarize these studies based on the developmental age of rodents at the time of analyzing the eCB system: childhood, adolescence, or adulthood.

eCB alterations induced by early life stress in childhood. A few studies have investigated the effects of early life MD on the eCB system in childhood. In 2008, Llorente et al. exposed rats to MD at PND 9 and reported elevated levels of 2-AG in the hippocampus in male rats at PND 13, which was reversed with OMDM-2, an eCB reuptake inhibitor. They reported no changes in the levels of AEA in MD rats.[46] Using the same paradigm, Suárez et al. investigated the effects of MD at day 9 on the eCB system in rats at PND 13, focusing on the CB1R and CB2R in the hippocampus (dentate gyrus,

CA1, and CA3). The results of the study showed that MD animals had a significant decrease in CB1R immunoreactivity and an increase in CB2R immunoreactivity in the hippocampus.[47] To understand the underlying mechanisms, in another study, Suárez et al. analyzed the enzymes involved in 2-AG metabolism, diacylglycerol lipase (DAGL) and MAGL, in the hippocampus at PND 13 of rodents with MD at PND 9. In this study, they found that MD induced a significant increase in DAGL immunoreactivity (the enzyme that produces 2-AG) in the CA3 and a significant decrease in MAGL immunoreactivity (the enzyme that degrades 2-AG) in hippocampal CA3 and CA1 areas.[48] Increasing levels of DAGL and decreasing levels of MAGL in hippocampus could explain the elevated levels of 2-AG and possibly downregulation of CB1R in hippocampus of young rodents with early life adversity. It is important to note that these studies all analyzed the eCB system on PND 13, only four days after the occurrence of MD. In the next section, we describe other studies that investigated eCB system of MD rodents during adolescence and adulthood.

eCB alterations induced by early life stress in adolescence. Marco et al. investigated the effects of MD (at PND 9) on eCB system gene expression in adolescent rats (PND 46), including the expression of genes encoding for CB1R and CB2R, TRPV1 and GPR55, the major enzymes of synthesis, N-acyl phosphatidyl-ethanolamine phospholipase D (NAPE-PLD) and DAGL, and degradation, FAAH and MAGL, in the frontal cortex, ventral and dorsal striatum, dorsal hippocampus, and amygdala. The results showed that MD increased the genetic expression of all eCB genes in the frontal cortex in adolescent males, and in the hippocampus in adolescent females.[49] In another study, Portero-Tresserra et al. exposed mice to maternal separation from PND 2 to PND 16 (4–8 h/day) and weaned them at PND 21 Maternal Separated- Early weaned (MSEW) and investigated their eCB system at PND 41. The results demonstrated that mice with MSEW showed deceased levels of AEA in the striatum and decreased levels of 2-AG in the PFC compared to the control group.[50]

eCB alterations induced by early life stress in adulthood. Romano-López et al. investigated the effects of maternal separation (2×3 h/day separation from PND 2 to PND 15) on the eCB system in adulthood (PND 75) and reported that adult rats had a significant decrease in CB1R in frontal cortex and a significant increase in ventral striatum, but no difference was found in the hippocampus.[51] Using the same procedure in another study (MD at PND 2–15, eCB system analysis at PND 100), Romano-López et al. reported lower presentation of MAGL and FAAH in the nucleus accumbens (NAcc) but found no alterations in the frontal cortex.[52] In another study, Llorente-Berzal et al. exposed neonatal rats to MD for 24 h at PND 9 and investigated the eCB system in adulthood (PND 85). As a result of MD in childhood, adult rats exhibited higher levels of CB1R expression in the substantia nigra (both males and females), whereas CB1R had lower function in NAcc, PFC, and hypothalamus, as well as lower expression in thalamus in males. Notably, CB1R activity (males) and expression (females) was increased in the cerebellum.[53] Overall, these studies demonstrate long-lasting effects of early life adversity on the eCB system in adulthood. However, the studies have some inconsistencies: decreased levels of CB1R are reported in frontal cortex, PFC, NAcc, and hypothalamus, which also fit well with decreased expression of MAGL and FAAH in NAcc and also result in increased levels of AEA and 2-AG. On the other hand, increased levels of CB1R were also seen in ventral striatum, substantia nigra, and cerebellum.

Taken together, it appears that the effects of early life trauma on the eCB system are different in each developmental period, despite some inconsistencies. Table 1 summarizes these studies and Figure 2 illustrates the summary of developmental effects of early life trauma.

Chronic Unpredictable Mild Stress. Another factor that modulates the effects of trauma on the eCB system is the chronicity of the trauma and its accumulative effects. Exposure to several mild and moderate traumatic experiences which occur on a daily basis such as work-related stressors or marital issues have long-lasting severe adverse consequences on mental health.

Chronic unpredictable stress (CUS) is an animal model of repetitive stressors, during which rodents are exposed to a series of mild, but unpredictable, different stressors such as swimming, cage rotations (social stress), social isolation with damp bedding, food and/or water deprivation, physical restraint, strobe light exposure, cage soiling with water, group housing in a confined space, intermittent lighting, reversal of light/dark cycle, cage tilting to 45°, and exposure to loud white noise. None of these stressors are considered severe, but when rodents are exposed to these stressors 8–12 h every day for about three weeks, they show decreased responsiveness to rewarding stimuli such as food and weight loss, enhanced fearfulness, impaired sleep, and decreased self-care.[56] Moreover, CUS induces cognitive impairments, including perseveratory behavior and impairments in extinction in a variety cognitive tasks, without affecting acquisition of learning.[57] CUS enhances hippocampal-dependent episodic fear memories, which in turn increases the susceptibility of developing PTSD. These conditions improve with CB1 receptor agonists, which suggests the potential role of eCB system deficiency in

Table 1. Animal and human studies investigating the impacts of childhood trauma on the eCB system.

References	Subject type (Sample size if provided)	Description of trauma	Age of trauma	eCB System markers	Brain region (if applicable)	Findings
Llorente et al.[46]	Wistar Albino rats of both sexes	24 h of maternal deprivation	Trauma: postnatal day 9 Tissue collection: postnatal day 13	Tissue AEA and 2-AG content	Hippocampus	↑ hippocampal 2-AG content in male rats only. No changes observed in tissue AEA content.
Suárez et al.[47]	Wistar Albino rats of both sexes (n = 28)	24 h of maternal deprivation	Trauma: postnatal day 9 Tissue collection: postnatal day 13	CB1 and CB2 receptor expression	DG, CA1, and CA3 regions of the hippocampus	Significant ↓ in CB1 immunoreactivity in CA1 and CA3, and significant ↑ in CB2 immunoreactivity throughout the hippocampus among stressed rats of both sexes. Female rats demonstrated higher CB1 immunoreactivity in CA3 and lower CB2 immunoreactivity in the dentate gyrus compared to male rats.
Suárez et al.[48]	Wistar Albino rats of both sexes (n = 28)	24 h of maternal deprivation	Trauma: postnatal day 9 Tissue collection: postnatal day 13	Activity and expression of enzymes involved in 2-AG synthesis (DAGL) and degradation (MAGL)	Hippocampus	Maternal deprivation resulted in a significant ↑ in DAGL immunoreactivity in the CA3, and a significant ↓ in MAGL immunoreactivity in CA1 and CA3 hippocampal regions in both male and female rats. ↓ of MAGL mRNA expression in male rats only.
Marco et al.[49]	Male and female offspring of Albino Wistar rats	24 h of maternal deprivation	Trauma: postnatal day 9 Tissue collection: postnatal day 46	eCB system gene expression	Frontal cortex, ventral and dorsal striatum, dorsal hippocampus, and amygdala	↑ genetic expression of all eCB system genes in the frontal cortex of adolescent males and in the hippocampus of adolescent females.
Portero-Tresserra et al.[50]	Male C57BL/6 mice offspring (n = 9–10 per group)	Maternal separation (4–8 h/day)	Trauma: postnatal days 2– 16 Tissue collection: postnatal day 41	Tissue AEA and 2-AG	Striatum Prefrontal cortex	↓ levels of AEA in the striatum and ↓ levels of 2-AG in the prefrontal cortex.

(continued)

Table 1. Continued.

References	Subject type (Sample size if provided)	Description of trauma	Age of trauma	eCB System markers	Brain region (if applicable)	Findings
Romano-López et al.[51]	Male Wistar rat pups (n = 8)	Maternal separation (two 3-h periods daily) for 14 days	Trauma: Postnatal days 2–16 Tissue collection: postnatal day 75	Cannabinoid receptors 1 and 2 in adulthood	Frontal cortex Ventral striatum Hippocampus	↑ CB1 expression in the ventral striatum and ↓ CB1 expression in the frontal cortex in stress-exposed rats. No difference in CB1 expression within the hippocampus between stress-exposed and non-stressed rats.
Romano-López et al.[52]	Male Wistar rat pups (n = 10 in each group)	Maternal separation (two 3-h periods daily) for 14 days	Trauma: Postnatal days 2 to 15. Tissue collection: postnatal day 100	MAGL and FAAH (eCB degrading enzymes) activity in adulthood	Frontal cortex Nucleus accumbens	Adult stress-exposed rats were found to have significantly ↓ FAAH and MAGL expression in the nucleus accumbens, but not in the frontal cortex.
Llorente-Berzal et al.[53]	Male and female Wistar rats	24 h of maternal deprivation	Trauma: Postnatal day 9 Tissue collection: postnatal day 85	Cannabinoid receptor expression and function in adulthood	Ventral tegmental area Nucleus accumbens Amygdala Hippocampus PFC Caudate putamen Substantia nigra Globus pallidus Thalamus Hypothalamus Periaqueductal gray Cerebellum	↑ levels of CB1R expression in the substantia nigra in male and female rats. ↓ CB1R function in the NAcc, PFC, and hypothalamus and ↓ CB1R expression within the thalamus of male rats only.
Koenig et al.[54]	Mothers exposed to childhood trauma and their newborns (n = 76 mothers, n = 37 newborns), and mothers without childhood trauma and their newborns (n = 74 mothers, n = 55 newborns)	Exposure to childhood trauma	Age of childhood trauma-exposed participants: 33.14 Mean age of control mothers: 32.04	Hair eCB ligands	Not applicable	↑ 1-AG and ↓ levels of SEA in hair of mothers with childhood trauma. Newborns of mothers with childhood trauma had ↑ levels of 1-AG and OEA.

(continued)

Table 1. Continued.

References	Subject type (Sample size if provided)	Description of trauma	Age of trauma	eCB System markers	Brain region (if applicable)	Findings
Schaefer et al.[55]	Patients with PTSD and history of childhood sexual abuse (n = 21), patients with borderline personality disorder (n = 26), and healthy controls (n = 30)	Childhood sexual abuse	Mean age PTSD group: 36.6 Mean age BPD group: 27.3 Mean age healthy controls: 31.5	Serum levels of endocannabinoids	Not applicable	↑ 2-AG and AEA in individuals with BPD. ↑ OEA in PTSD with history of childhood trauma.

AEA: N-arachidonylethanolamine (also called anandamide); 2-AG: 2-arachidonoylglycerol; CB1: cannabinoid receptor 1; CB2: cannabinoid receptor 2; DG: dentate gyrus; CA1: *Cornu Ammonis 1*; CA3: *Cornu Ammonis 1*; DAGL: diacylglycerol lipase; MAGL: monoacylglyceride lipase; eCB: endocannabinoid; FAAH: Fatty acid amide hydrolase; NAcc: nucleus accumbens; PFC: prefrontal cortex; SEA: N-stearoylethanolamide; 1-AG: 1-arachidonoylglycerol; OEA: N-oleoylethanolamide; PTSD: posttraumatic stress disorder; BPD: borderline personality disorder.

PTSD vulnerability in individuals with history of exposure to chronic stress.[58]

Accumulating evidence reliably demonstrate eCB alterations as a result of CUS. Robust reductions in both 2-AG content (about 40% reduction) and CB1R density (about 50% reduction) within the hippocampus, but not limbic forebrain, have been reported in rats after 21 days of CUS.[57] In this study, stressed animals showed perseveratory behaviors and impairments in extinction learning similar to CB1R knocked out animals, which was completely reversed by pre-treatment with CB1R agonist injection.[57] Another study found significant decreased levels of AEA in all brain areas as well as reduction of CB1R in the hippocampus, hypothalamus and ventral striatum, but an increase in CB1R in PFC (57). Reduction of CB1R has been also reported in nucleus accumbens after five to six weeks of CUS.[59] CUS-induced symptoms improve with inhibitors of eCB degrading enzymes such as FAAH (degrading AEA)[60] and MAGL (degrading 2-AG).[61]

In an important study, Reich et al. investigated the effects of cannabinoid receptor agonists in rodents after three weeks of daily CUS.[62] The findings of the study demonstrated that exogenous activation of CB1R by WIN in stressed animals resulted in a ~135% increase in excitatory neurotransmission, whereas CB1R activation in nonstressed animals induced a ~30% decrease in glutamatergic neurotransmission. They also reported that during the blockade of GABA neurotransmission, CB1R activation yielded a ~35% decrease glutamatergic neurotransmission in stressed animals, which suggests that CUS does not directly affect glutamatergic neurotransmission but sensitizes CB1R function on GABAergic terminals, leading to less inhibition and an increase in excitatory neurotransmission in stressed animals.[62]

Lomazzo et al. investigated the epigenetic changes in mice exposed to CUS and found that CB1R expression is decreased in non-GABAergic low-expressing CB1R neurons in the cingulate cortex. They reported anxiety-like and depressive-like behaviors in CUS animals and demonstrated that FAAH inhibitors improve the anxiety-like behaviors.[63] The aforementioned studies are summarized in Table 2.

PTSD Animal Models. Exposure to a single severe traumatic event in adulthood is another form of trauma that has been studied in animal research as a potential model for PTSD.

Shock and reminder stress. The formation of fear memory after a traumatic event and impairment in its extinction are at the core of development of PTSD symptoms. To investigate the underlying mechanism, severe foot shock, followed by situational reminders, has been shown to induce long-term impairment of fear

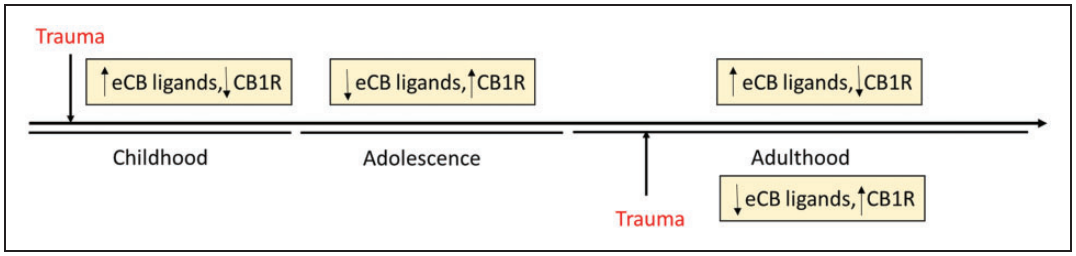

Figure 2. Effects of childhood and adulthood trauma on eCB in childhood, adolescence, and adulthood. eCB: endocannabinoid; CB1R: cannabinoid receptor 1.

extinction and enhancement of startle response and is used as an animal model of PTSD.[11] Using the same model, Korem et al. found decreased levels of AEA in NAcc in traumatized rats. They also reported that microinjection of CB1R agonist, WIN55,212-2, into NAcc facilitates the extinction response in shocked rats.[65]

In a recent study, Fidelman et al. used this model to investigate the effects of FAAH inhibitor, URB597, and synthetic cannabinoid, WIN 5212-2, on PTSD-like symptoms. The results of the study showed significant improvement of startle response and fear extinction after three weeks administration of FAAH inhibitors, whereas WIN only reversed the startle response, and did not affect fear extinction. Both compounds affected PTSD-like symptoms through CB1R and were blocked with the administration of CB1R antagonist. Moreover, URB597, but not WIN, normalized the CB1R upregulation in the basolateral amygdala and CA1.[11] In another study using the same model, administration of FAAH inhibitor, AM3506, before extinction training decreased fear response during a retrieval test. Moreover, anandamide levels in the basolateral amygdala were increased by extinction training and augmented by AM3506. These effects were blocked by intra-amygdala infusion of CB1R antagonist.[38]

Single prolonged stress. Single prolonged stress (SPS) model was also used as an animal model of PTSD. Using this model, Zer-Aviv and Akirav exposed rodents to restraint, forced swim, and sedation and investigated their eCB system after 10 days. The results demonstrated an increase in the levels of CB1R in amygdala, PFC, and hippocampus. Moreover, FAAH inhibitor, URB597, was able to normalize the SPS-induced upregulation in CB1R levels in the amygdala, PFC, and hippocampus in males, and in the amygdala and PFC, but not hippocampus, in females.[66]

Life-threatening trauma. Exposure to 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), a chemical constituent of fox feces, produces robust anxiety-like behaviors in rats, which last for weeks and has been used as a model of posttraumatic long-term anxiety state. In a recent study, Danandeh et al. used this model to investigate the effects of FAAH inhibitor, URB597, on these anxiety-like behaviors, and reported that the administration of URB597 increases the brain levels of AEA and effectively prevents the development of posttraumatic anxiety-like behaviors in a CB1R-dependent manner.[13] Using the same model, Lim et al. reported increased levels of 2-AG in the amygdala in traumatized rodents with anxiety-like behaviors, which effectively improved with the administration of MAGL inhibitor after the trauma.[12] Table 3 summarizes studies exploring eCB system alterations following adulthood trauma.

Clinical Studies

There has been increasing attention to the role of eCB alterations in PTSD symptomatology in humans over the past decade and an increasing number of studies have been published on alterations in the eCB system among individuals diagnosed with PTSD over the past few years. However, most of these studies measured peripheral levels of eCB ligands and have reported inconsistent results. Moreover, these studies have been mainly designed to investigate the eCB system in individuals with PTSD and did not differentiate the effects of childhood or chronic trauma exposure from one single severe trauma in adulthood. Next, we will summarize these studies, based on our preclinical model of differentiated developmental and accumulative effects of trauma on the eCB system.

Childhood Trauma. We found only one study that specifically investigated the effects of childhood trauma on peripheral eCB system markers. In this study, Koenig et al. assessed the association between history of childhood trauma in 142 pregnant mothers with the concentration of eCBs in their hair samples. The results showed that hair of mothers with childhood trauma contained significantly higher levels of 1-AG and lower levels of SEA. They also reported that history of more severe childhood trauma was associated with the lower levels of SEA levels.[54]

Another study that we would like to include in this section is an investigation of peripheral eCB ligands in individuals diagnosed with BPD and PTSD with history of childhood sexual abuse. In this study, Schaefer et al.

Table 2. Animal and human studies investigating the impacts of chronic unpredictable mild stress on the eCB system.

References	Subject type (Sample size)	Description of Trauma	Age of trauma	eCB System markers	Brain Region (if applicable)	Findings
Hill et al.[55]	Male Long-Evans rats n = 4–6 (biochemical assays) n = 9–10 (behavioral analysis)	21 days of CUS	70-day-old	2-AG, AEA tissue content CB1 receptor binding assays and Western blots Task performance on the Morris water maze	Hippocampus Limbic forebrain	↓ in CB1 receptor protein and receptor binding in the hippocampus in CUS animals. ↓ in 2-AG content in hippocampus of stressed animals. Learning deficits and perseverative behavior in CUS exposed rats during the Morris water maze.
Hill et al.[64]	Male Long-Evans rats n = 7–8 (eCB content) n = 4–5 (CB1 receptor binding density)	21 days of CUS	70-day-old	CB1 receptor binding site density	Hippocampus Hypothalamus Ventral striatum Prefrontal cortex Amygdala Midbrain	Significantly ↓ AEA levels in all brain areas. Reduced CB1 receptor binding density in hippocampus, hypothalamus, and ventral striatum. ↑ CB1 receptor binding density in the prefrontal cortex.
Wang et al.[59]	Male C57BL/6J mice	5–6 weeks of CUS	8–10 weeks of age	eCB/CB1 receptor-mediated synaptic plasticity	Nucleus accumbens	Downregulation and deficiency of eCB/CB1 receptor-mediated responses in the nucleus accumbens following CUS.
Bortolato et al.[60]	Male Wistar Rats (n = 120)	5 weeks of CUS	Not specified (weight at start of protocol: 200 g)	Body weight, sucrose intake, and brain FAAH activity and endocannabinoid content was analyzed	Midbrain Prefrontal cortex Hippocampus Striatum Thalamus	Stressed rats showed ↓ body weight gain and sucrose intake, which normalized s/p FAAH inhibitor treatment. No significant changes in AEA levels in any of the 5 brain regions following CUS. Small ↑ in 2-AG in the thalamus, no significant changes in other regions. FAAH inhibitor treatment ↑ AEA levels in midbrain, striatum, and thalamus in both stressed and nonstressed rats.

(continued)

Table 2. Continued.

References	Subject type (Sample size)	Description of Trauma	Age of trauma	eCB System markers	Brain Region (if applicable)	Findings
Zhang et al.[61]	Male C57BL/J mice	5 weeks of CUS	8–10 weeks of age	Hippocampal neurogenesis Long-term potentiation activity	Hippocampus	CUS impaired hippocampal neurogenesis and impaired long-term potentiation within the dentate gyrus of the hippocampus. Treatment with MAGL inhibitor prevented these changes in stressed mice.
Reich et al.[58]	Male Sprague-Dawley Rats	3 weeks of daily CUS	40–45 days old	Fear memory conditioning and extinction	Not applicable	CUS enhanced hippocampal-dependent trace fear conditioning. CB1 receptor agonist prevented enhanced fear conditioning and promoted long-term fear extinction.
Reich et al.[62]	Male Sprague-Dawley rats Stress (n = 20) Nonstressed (n = 12)	3 weeks of daily CUS	40–45 days old	eCB-regulated glutamatergic neurotransmission	Hippocampal area CA1	CB1 agonist resulted in ↑ in excitatory neurotransmission in stressed rats, while in non-stressed there was a ↓ in excitatory neurotransmission.
Lomazzo et al.[63]	Male CB57BL/6J mice	11 weeks of CUS	6 weeks	Neuropeptide-Y expression and receptor signaling, CB1 receptor expression and epigenetic modifications within the neuropeptide Y system and the CB1 gene	Cingulate cortex	↓ neuropeptide-Y expression in mice exposed to CUS. ↓ neuropeptide-Y receptor signaling, and ↓ CB1 receptor expression within the cingulate cortex. CUS was also associated with ↓ histone acetylation of Npy and CB1 genes.

CUS: chronic unpredictable stress; 2-AG: 2-arachidonoylglycerol; AEA: N-arachidonylethanolamine (also called anandamide); CB1: cannabinoid receptor 1; eCB: endocannabinoid; FAAH: fatty acid amide hydrolase; MAGL: monoacylglyceride lipase; N/A: not applicable.

Table 3. Animal and human studies investigating the impacts of adulthood prolonged stress or trauma on the eCB system.

References	Subject type (Sample size)	Description of Trauma	Age of trauma	eCB System markers	Brain Region (if applicable)	Findings
Fidelman et al.[11]	Male Sprague-Dawley rats	Inescapable footshock	Postnatal day 67	Tissue AEA and 2-AG content FAAH and MAGL activity	Basolateral amygdala Hippocampus	FAAH inhibitor administration improved startle response and fear extinction in rats exposed to footshock. ↓ AEA in the BLA and CA1 1 h following footshock exposure, no changes in serum AEA levels. Serum 2-AG was ↓ in shocked rats, no significant change in the BLA or CA1. ↑ FAAH and MAGL activity in the CA1 (but not the BLA).
Korem et al.[65]	Male Sprague-Dawley rats	Inescapable footshock	60 days	Tissue AEA, 2-AG, PEA, OEA, and 2-OG content	Nucleus accumbens	↓ AEA, 2-OG, and OEA content in the nucleus accumbens of rats exposed to shock and reminders.
Gunduz-Cinar et al.[38]	Male 129S1/Sv1mJ mice	Footshock	8- to 12-week-old mice	Tissue AEA and 2-AG content	Amygdala	↑ AEA levels in the BLA after extinction training, which was augmented by FAAH inhibition.
Zer-Aviv and Akirav[66]	Male and female Sprague-Dawley rats (n = 9–10 in each experiment group)	Single prolonged stress (restraint stress followed by forced swim task and sedation)	Adult (weighing 260–300 g)	CB1R expression	Infralimbic-PFC BLA CA1 Ventral subiculum	↑ CB1R in BLA, IL-PFC, and hippocampus. Increase was normalized by FAAH inhibition in males, and within the BLA and IL-PFC, but not hippocampus, in females.

(continued)

Table 3. Continued.

References	Subject type (Sample size)	Description of Trauma	Age of trauma	eCB System markers	Brain Region (if applicable)	Findings
Danandeh et al.[13]	Male Sprague-Dawley rats (n = 184)	2,5-dihydro-245-trimethylthiazoline (chemical constituent of fox feces) exposure for 20 min	8–9 weeks old	FAAH activity and tissue AEA, PEA, and OEA content	Whole brain	FAAH inhibition prevented anxiety-like behaviors following trauma event. CB1R antagonist blocked anxiolytic-like effects of FAAH inhibition. FAAH inhibition ↑ whole brain levels of all FAAH substrates (including AEA).
Lim et al.[12]	Male Sprague-Dawley rats (n = 6–8 rats per experimental group)	2,5-dihydro-245-trimethylthiazoline (chemical constituent of fox feces) exposure for 10 min	8–9 weeks old	Tissue AEA and 2-AG content MAGL activity	Hypothalamus Amygdala Dorsal and ventral hippocampus Cerebellum	↑ 2-AG in the amygdala at 24 h following TMT exposure (remained elevated for next 13 days) Anxiety-like behavior observed in rats following stressor was improved with MAGL inhibition.
Hill et al.[67]	Individuals with PTSD (n = 24) or no PTSD (n = 22) with physical proximity to World Trade Center at the time of 9/11 attack	World Trade Center attack (9/11)	Not applicable	Plasma eCB markers 4 to 6 years following the trauma	Not applicable	↓ plasma 2-AG levels in individuals meeting diagnostic criteria for PTSD. No significant group differences for plasma cortisol or AEA.
Yi et al.[68]	Healthy male volunteers (n = 6)	520 days of extreme social isolation and confinement (simulation of a flight to Mars)	Mean age: 33	Plasma eCB markers taken at days 360, 410, and day 51	Not applicable	Significant ↓ in the blood levels of 2-AG, with no changes in serum AEA levels.
Wilker et al.[69]	Rebel war survivors from Northern Uganda with PTSD (n = 38) compared to rebel war survivors without current or lifetime PTSD (n = 38)	Exposure to the war between the rebel group Lord's Resistance Army and the Ugandan Governmental forces from Northern Uganda	Mean age of PTSD group: 30.89 Mean age of control group: 31	Hair PEA, OEA, and SEA content	Not applicable	↓ in hair OEA concentration in individuals with PTSD. Negative relationship between all eCB levels and the severity of PTSD symptoms.

(continued)

Table 3. Continued.

References	Subject type (Sample size)	Description of Trauma	Age of trauma	eCB System markers	Brain Region (if applicable)	Findings
Neumeister et al.[70]	Individuals with PTSD (n = 25), trauma exposed individuals without PTSD (n = 12), and healthy individuals without trauma history (n = 23)	Non-combat trauma (physical assault, motor vehicle accident, witnessed suicide)	Mean age of PTSD: 32.2 Mean age of trauma controls: 29.7 Mean age of health controls: 32.1	CB1 receptor availability Peripheral levels of AEA, 2-AG, OEA, PEA PET scanner with CB1 selective radioligand	Brain-wide	↑ brain-wide availability of CB1R in the PTSD group. ↓ peripheral levels of AEA in individuals with PTSD compared to the other groups.
Pietrzak et al.[71]	Trauma exposed participants (n = 16) and healthy, non-trauma exposed controls (n = 4)	Various index traumas (sexual assault, witnessed death, physical assault, motor vehicle accident)	Mean age: 33.3	Plasma AEA levels High resolution research tomograph PET scanner with CB1 selective radioligand	Amygdala	↑ CB1R availability in amygdala in those with history of trauma was associated with increased attentional bias to threat. AEA levels were negatively associated with CB1R availability, and lower AEA levels were associated with ↑ attentional bias to threat.
Hauer et al.[72]	Trauma exposed individuals with PTSD (n = 10), trauma exposed individuals with no PTSD (n = 9), and healthy controls (n = 29)	Trauma-exposed participants were refugees with history of persecution, war, and torture experiences	Mean age PTSD: 33.8 Mean age trauma-exposed, no PTSD: 33.6 Mean age, healthy controls: 33.5	Peripheral eCB levels (AEA, 2-AG, PEA, OEA, SEA, OLDA)	Not applicable	↑ levels of AEA, 2-AG, OEA, PEA, and SEA in individuals with PTSD.

[2]-AG: 2-arachidonoylglycerol; AEA: N-arachidonylethanolamine (also called anandamide); FAAH: fatty acid amide hydrolase; MAGL: monoacylglyceride lipase; BLA: basolateral amygdala; PEA: N-palmitoylethanolamide; OEA: N-oleoylethanolamide; SEA: N-stearoylethanolamide; 2-OG; PFC: prefrontal cortex; CA1: Cornu Ammonis 1; IL-PFC: interleukin PFC; CB1R; cannabinoid receptor 1; TMT: 2,5-dihydro-2,4,5-trimethylthiazoline; PTSD: posttraumatic stress disorder; eCB: endocannabinoid; CB1: cannabinoid receptor 1; PET: positron emission tomography scanN-oleoyldopamine; OLDA: N-oleoyldopamine.

enrolled 21 patients with PTSD, 26 patients with BPD, and 30 matched healthy controls. They measured peripheral levels of eCB and found increased levels of 2-AG and AEA in individuals with BPD and increased levels of OEA in PTSD (with history of childhood trauma).[55]

These two studies are consistent with animal studies on eCB system alterations in rodents with early life trauma (reviewed earlier). Taken together, it seems that if trauma happens early in life, significant changes in eCB are present in adulthood, with mainly increased levels of eCB ligands (1-AG, 2-AG, or AEA) and decreased presentations of CB1R.

Chronic Repetitive Unpredictable Mild Stress in Adulthood. Currently, aside from chronic childhood abuse studies noted earlier, there are no other clinical studies that examine the effects of chronic (prolonged) repetitive unpredictable mild stress on eCB system.

Severe Trauma in Adulthood. There are a few studies published on the eCB system in individuals exposed to a major single trauma or prolonged severe stress in adulthood.

Single severe trauma. Hill et al. investigated the peripheral eCB markers (2-AG, AEA, OEA, and PEA), in individuals (n = 46) exposed to the World Trade Center (WTC) Attack (9/11), four to six years after the trauma. The results of the study showed reduced plasma 2-AG levels in individuals meeting diagnostic criteria for PTSD compared to those who were exposed to the trauma but did not meet diagnostic criteria for PTSD. The association between PTSD diagnosis and reduced 2-AG levels remained significant after controlling for the stress of exposure to the WTC collapse, gender, depression, and alcohol abuse.[67]

In addition to the peripheral levels of eCB ligands, CB1R alterations in individuals with history of trauma and PTSD have been investigated using CB1-selective radioligand [11C]OMAR and PET imaging evaluations. Neumeister et al. measured CB1 receptor availability as well as peripheral levels of AEA, 2-AG, OEA, and PEA in individuals with PTSD (n = 25), trauma-exposed individuals without PTSD (n = 12) and healthy individuals without history of trauma (n = 23). The results of the study showed increased brain-wide availability of CB1R and reduced peripheral levels of AEA in individuals with PTSD compared to the other two groups.[70] Using the same methods, Pietrzak et al. reported that increased availability of CB1R in amygdala in individuals with history of trauma was associated with increased attentional bias to threat and increased severity of threat which was measured using a dot-probe task. In this study, they found that lower peripheral AEA levels were associated with higher levels of attentional bias to threat.[71]

Prolonged severe trauma. An important study on the effects of severe prolonged adulthood trauma on the eCB system is conducted by Yi et al. on six healthy individuals who were exposed to 520 days of extreme social isolation and confinement as a simulated model of a flight to Mars (Mars520). The results of the study showed a significant decrease in the blood levels of 2-AG (present after 360 days) with no significant changes in the AEA levels compared to the baseline levels.[68]

In another study, eCB concentrations in hair samples of 38 rebel war survivors from Northern Uganda with PTSD was compared with 38 healthy rebel war survivors without current and lifetime PTSD. The authors reported they could not reliably measure the AEA and 2-AG concentrations but found decreased concentrations of OEA in individuals with PTSD and a negative relationship between all eCB levels and the severity of PTSD symptoms.[69]

In contrast with the aforementioned studies, another study was performed to investigate the peripheral eCB system markers in 19 trauma-exposed individuals (10 with PTSD and 9 without PTSD) compared to healthy controls. Trauma-exposed individuals in this study were refugees, and most of them (84.2%) had history of persecution, war, and torture experiences in their early or late adulthood. The results of analyzing peripheral eCB in these individuals showed higher levels of AEA, 2-AG, OEA, SEA, and PEA in individuals with PTSD compared to trauma without PTSD and controls. However, it is important to note that individuals with PTSD significantly had experienced higher numbers of traumatic events, specially torture and war-related events, compared to the trauma without PTSD group. Moreover, the participants in this study had heterogenous race/ethnicity, with some comorbid psychiatric disorders including depression (positive in 9 out of 19 trauma-exposed participants), or other medical disorders such as rheumatic disorder or hepatitis B (4 out of 19 trauma-exposed individuals). Although authors conducted several comparison studies and reported no effects of these comorbidities or psychiatric medications on the eCB levels, the sample size of each group was small.[72] These comorbidities and heterogenicity in the amount and type of trauma may explain the contradictory results compared to the two other studies.

Conclusion

Long-lasting eCB system alterations have been consistently reported both in animal and human studies. However, it is critically important to consider the developmental and accumulative effects of trauma when investigating the nature and direction of these observed changes. Although some of the inconsistencies are explained with different brain regions of study or technical differences in measuring the markers, the persistent effects of trauma on the eCB system seem to be different when the trauma is

experienced during childhood compared to adulthood. Animal models of childhood trauma demonstrate different effects of trauma on the eCB system, depending on the developmental stage when investigating the eCB system. As illustrated in Figure 2, childhood trauma results in increased levels of eCB ligands and decreased levels of CB1R in childhood, decreased levels of ligands and increased levels of CB1R in adolescence, and increased levels of eCB ligands and decreased levels of CB1R in adulthood. On the other hand, the nature of childhood trauma in humans is usually chronic and repetitive. CUS in rodents induces downregulation of CB1R as well as lower levels of eCB ligands. There is only one human study on eCB system alterations in individuals with history of childhood trauma, and the results are consistent with animal models, with increased levels of eCB ligands. Similarly, another study on individuals with BPD and PTSD with history of childhood trauma reported increased levels of 2-AG and AEA in individuals with BPD and increased levels of OEA in PTSD with history of childhood trauma. Taken together, it seems that both preclinical and clinical studies consistently demonstrate increased levels of eCB ligands and decreased levels of CB1R in adults with history of childhood trauma and PTSD.

In contrast, adulthood severe trauma induces decreased levels of eCB ligands and increased presentation of CB1R in both animal models and the majority of human studies. It seems that based on the developmental age when exposure to trauma occurs, long-lasting induced eCB alterations shifts the eCB system into opposite directions. To date, there is no available preclinical or clinical study investigating the effects of developmental age of subjects at the time of trauma on the long-lasting induced eCB system alterations. It is important to note that though in this review we categorized the studies based on the developmental age of subjects at the time of trauma to differentiate the eCB alterations induced by childhood versus adulthood trauma, several other factors may contribute to these differences such as type or duration of trauma or technical differences in research methodology.

More studies are needed to compare the effects of childhood and adulthood trauma, with or without PTSD presentations, on the eCB system. These studies would have important clinical implications, not only for individuals with trauma and PTSD who commonly have comorbid recreational cannabis use, and medical marijuana users with PTSD being one of its main indicators but also for studies investigating the potential therapeutic use of cannabinoids and eCB enhancers in PTSD treatment.

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