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Stress Regulates Endocannabinoid-CB1 Receptor Signaling

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Abstract

The CB1 cannabinoid receptor is a G protein coupled receptor that is widely expressed throughout the brain. The endogenous ligands for the CB1 receptor (endocannabinoids) are *N*-arachidonylethanolamine and 2-arachidonoylglycerol; together the endocannabinoids and CB1R subserve activity dependent, retrograde inhibition of neurotransmitter release in the brain. Deficiency of CB1 receptor signaling is associated with anhedonia, anxiety, and persistence of negative memories. CB1 receptor-endocannabinoid signaling is activated by stress and functions to buffer or dampen the behavioral and endocrine effects of acute stress. Its role in regulation of neuronal responses is more complex. Chronic variable stress exposure reduces endocannabinoid-CB1 receptor signaling and it is hypothesized that the resultant deficiency in endocannabinoid signaling contributes to the negative consequences of chronic stress. On the other hand, repeated exposure to the same stress can sensitize CB1 receptor signaling, resulting in dampening of the stress response. Data are reviewed that support the hypothesis that CB1 receptor signaling is stress responsive and that maintaining robust endocannabinoid/CB1 receptor signaling provides resilience against the development of stress-related pathologies.

1. Introduction: Stress

Physical and psychological threats to the well-being of an individual induce a pattern of physiological responses that are designed to cope with the immediate stress, avoid future threats, and facilitate restoration of homeostasis. Stress exposure results in a broad and significant impact on physiological and psychological function designed to increase chances for escape, and for survival if an injury does occur. As a result of sympathetic nervous system activation, stress exposure increases heart rate, blood pressure and blood flow to muscles. Activation of the sympathetic nervous system (SNS) activation also promotes a pro-inflammatory environment in the periphery and brain [1]. For example, exposure of healthy males to social stress increases circulating T cells and concentrations of pro-inflammatory cytokines, including interleukin-1β [2]. In addition, stress exposure activates the hypothalamic-pituitary-adrenocortical (HPA) axis, and thereby increases circulating

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glucocorticoid concentrations. The glucocorticoids (cortisol in humans and corticosterone (CORT) in rodents) are steroids released by the adrenal cortex that produce physiological changes across a time range from seconds to hours through non-genomic and genomic mechanisms [3, 4]. Glucocorticoids affect memory and mood and alter the cardiovascular, metabolic and immune systems largely through effects on glucocorticoid receptors (GR).

Although these stress responses can be life-preserving in the face of a threat, they are costly when triggered repeatedly. In fact, chronic exposure to stress is a significant risk factor for psychiatric [5], metabolic [6], and functional pain disorders [7], as well as for neurodegeneration [8]. There is considerable individual vulnerability to the negative consequences of chronic stress. Resilience is associated with coping styles that enable individuals to maintain hedonia and optimism; to continue employing effective behavioral repertoires despite fear; and is associated with positive social interactions [9, 10]. On the other hand, hyperactive HPA responses to stress are associated with increased vulnerability to develop the negative consequences of chronic stress [11]. Importantly, coping styles and HPA axis responsivity are shaped by previous stress exposure and are particularly sensitive to early life stress [12].

2. Introduction: CB1 Receptor Mediated Endocannabinoid Signaling

2.1 The CB1 Cannabinoid Receptor

Δ^9-Tetrahydrocannabinol (THC) is the component of *cannabis sativa* that is responsible for the psychoactive effects of the plant [13]. The effects of THC on cognition and mood are mediated by its ability to act as an agonist of a G protein coupled receptor, named the CB1 cannabinoid receptor (CB1R) [14, 15]. The CB1R is present on many neuronal subtypes throughout the brain, and in peripheral nerves as well [16]. Very high density CB1R protein expression occurs in the cingulate gyrus, prefrontal cortex (PFC), hippocampus, cerebellum, basal ganglia and substantia nigra; moderate density is seen in the basal forebrain, amygdala, nucleus accumbens, periaqueductal gray and hypothalamus; and low density occurs in brain stem regions, primary motor cortex and thalamus [17]. The psychoactive effects of *cannabis sativa* in humans are completely antagonized by a selective antagonist of the CB1R [18].

Outside of the brain, CB1R are present in the dorsal horn of the spinal cord; specifically found on interneurons and axon terminals of descending inputs and peripheral afferents [17]. Primary sensory afferents also express the CB1R on terminals in innervated tissues [19]. Functional data indicate that sympathetic nerves express CB1R [20]; and CB1R are distributed throughout the neurons of the enteric nervous system [21].

Non-neuronal cells in the brain also express CB1R, including astrocytes [22], oligodendrocytes [23] and cells of the cerebral vasculature [24, 25]. Outside of the CNS, CB1R are expressed by circulating immune cells [26], adipocytes [27], hepatocytes [28], and adrenal cortex [29]. Thus, CB1R activation by exogenous or endogenous agonists has the potential to modify function of many organs, most particularly the brain.

2.2 The Endocannabinoids

In 1992, a low abundance member of the family of *N*-acylethanolamines (NAEs) was shown to function as an endogenous agonist of the CB1R [30]. This lipid, *N*-arachidonylethanolamine (AEA, also called anandamide), functions as a partial agonist of CB1R [31]. A second arachidonate, 2-arachidonoylglycerol (2-AG), was identified a short time later [32, 33]. 2-AG functions as a fully efficacious agonist of the CB1R [34]. Both AEA and 2-AG are considered endocannabinoids (eCBs), although their specific roles as regulators of CB1R signaling are not well understood [35].

AEA is synthesized from a minor phospholipid, *N*-arachidonyl-phosphatidylethanolamine (NAPE) [36], via several possible enzymatic processes [37]. The regulatory mechanisms of these synthetic pathways are not well understood. 2-AG is biosynthesized from diacylglycerol (DAG), through the actions of sn-1 specific DAG lipases (DAGLα and β) [38]. DAG is produced from phosphatidylinositol-bis-phosphate, which is metabolized by phospholipase C (PLC) to DAG and inositol triphosphate. DAGLα, which is thought to be the rate limiting step in 2-AG synthesis, is present on the postsynaptic membrane of neurons that make synaptic contact with neurons expressing the CB1R [39].

Two amidohydrolases have been identified that hydrolyze and thus, inactivate, AEA. The first, fatty acid amide hydrolase (FAAH), is present in the brain [40] and is an important regulator of brain concentrations of AEA as well as other NAEs [41, 42]. The second amidohydrolase, *N*-acylethanolamine-hydrolyzing acid amidase, hydrolyzes AEA in the periphery [43]. 2-AG is also inactivated by hydrolysis; more than 80% of the serine hydrolase-mediated metabolism of 2-AG in the brain is accomplished by monoacylglycerol lipase (MAGL) [44]. Another important serine hydrolase that metabolizes 2-AG is alpha-beta hydrolase 6 [45]. Both AEA and 2-AG are also substrates for cyclooxygenase 2 (COX2); the oxygenation of the eCBs by COX2 results in inactivation with respect to CB1R signaling [46].

2.3 Endocannabinoid Regulation of Synaptic Activity

There are considerable data supporting an important role for eCB/CB1R signaling in the regulation of synaptic plasticity. eCB signaling (ECS) underlies retrograde, activity-dependent, suppression of neurotransmitter release in many regions of the brain [47]. In brief, activation of CB1R on presynaptic terminals produce long- and short-term inhibition of vesicular transmitter release. The short term effects are mediated by CB1R-initiated, G protein-mediated inhibition of the opening of voltage operated calcium channels. The long term effects likely involve changes in the machinery regulating neurotransmitter release. As a result, the activation of CB1R on presynaptic terminals results in inhibition of neurotransmitter release for variable lengths of time.

Activation of presynaptic CB1R signaling occurs as a result of increased eCB mobilization. 2-AG is synthesized by post-synaptic neurons in response to several triggers, including receptors that activate PLC [48]; increased calcium concentrations, secondary to neuronal activity [47] and glucocorticoids (discussed in section 2.4.). The mechanisms and cellular site of AEA synthesis are not well understood. Since both 2-AG and AEA are highly

lipophilic, increased intracellular concentrations drive increased extracellular concentrations; in other words, synthesis and release are coupled. The eCBs can diffuse from the synthetic cells and inhibit neurotransmitter release from nearby neurons that express CB1R. CB1R are present on glutamatergic [49], GABAergic [50], serotonergic [51] and noradrenergic [52] axon terminals in the brain; thus ECS can regulate the release of the primary neurotransmitters involved in processing of stress and fear.

2.4. Glucocorticoids Alter Synaptic Activity via ECS

Glucocorticoids rapidly modulate glutamatergic [53, 54] and GABAergic [55] transmission in stress-sensitive brain regions through alteration in the release of neurotransmitters. Recent studies support the hypothesis that glucocorticoids modulate the release of glutamate and GABA as a result of enhanced ECS. In the hypothalamus, 2-AG concentrations are significantly elevated by restraint stress [56] and by CORT treatment [57]. Evidence suggests that glucocorticoids act through a membrane receptor to rapidly mobilize eCBs [58]. The consequence of ECS activation is to reduce the release of glutamate and, thus reduce excitatory drive onto corticotropin releasing hormone (CRH) neurons in the paraventricular nucleus (PVN) [58, 59]. This mechanism is hypothesized to contribute to glucocorticoid-mediated feedback inhibition of HPA axis activation at the level of the hypothalamus. Recent data also extend the glucocorticoid-eCB functional pair to regulation of excitatory transmission onto serotonergic neurons of the dorsal raphe [60]. Stress exposure also increases concentrations of 2-AG in the PFC and hippocampus. Glucocorticoid treatment of slices taken from PFC [61] and hippocampus [62] results in CB1R-mediated inhibition of GABA release.

These studies support the hypothesis that eCBs function as a second messenger for glucocorticoids in stress-responsive brain regions; in particular, allowing glucocorticoid presence to produce rapid changes in synaptic activity [63]. Available data indicate that glucocorticoids increase 2-AG concentrations; however, it seems that different glucocorticoid receptor sub-types and signaling cascades are employed, allowing for the specific pattern and time-course of eCB mobilization to be matched to the function of the glucocorticoids at a particular synapse. COX2 can oxidize both AEA and 2-AG [46] and its inhibition has been shown to increase ECS in the hippocampus [64, 65] and dorsal raphe [60]. There is evidence that COX2 is negatively regulated by glucocorticoids [66] supporting the possibility that glucocorticoids can regulate concentrations of the eCBs available to signal via alterations in the activity of COX2. This mechanism could be important for linking changes in inflammatory state with changes in synaptic activity.

3. Regulation of Mood, Cognition and Stress by CB1R Signaling

Preclinical and human data demonstrate that eCB/CB1R signaling is required for regulation of stress responses and mood. This conclusion is primarily support by studies in which eCB/CB1R signaling is reduced with pharmacologic antagonists or genetic deletion. CB1R blockade and deletion results in excessive [67, 68] and prolonged [61] activation of the HPA axis by stress. These data are in accord with the discussion in section 2.4 that ECS in several brain regions subserves glucocorticoid-mediated feedback inhibition in multiple brain regions.

Inhibition of eCB/CB1R signaling can result in increased anxiety. Rodents treated with a CB1R antagonist demonstrate increased anxiety-like behaviors [69]. Importantly, anxiety was a significant adverse effect in humans treated with a CB1R antagonist for metabolic disorder and obesity [70], and treatment of healthy humans prior to an experimental stress paradigm results in increased anxiety [71]. Reduced eCB/CB1R signaling also results in delayed and ineffective extinction of fearful memories in rodents [72]; is associated with reduced behavioral flexibility [73] and with anhedonia [74]. A subset of humans exposed to CB1R antagonists, particularly those with prior depressive symptoms, exhibited increased depressive symptoms, including suicidality [75]. Furthermore, women with depression exhibit significantly reduced 2-AG concentrations in the circulation [76], further evidence that hypoactive ECS is associated with negative affect.

These and other data strongly support the hypothesis that robust eCB/CB1R signaling is vital for appropriate stress responses and for the maintenance of emotional homeostasis, particularly in the face of chronic stress.

4. Regulation of ECS by Stress

The data described below support the role of ECS as a homeostatic mechanism that both inhibits unnecessary HPA axis activation through actions in the amygdala and promotes the recovery of the HPA axis to baseline after the threat has ended. While neither of these properties is absolutely required for the endocrine response to occur, loss of ECS would be expected to increase the "wear and tear" of stress on the brain because the stress response is activated with less provocation and remains in an active state for longer periods of time.

The ECS stress buffer is reduced by chronic stress exposure. Chronic stress is a significant risk factor for the development of psychopathology, and there are considerable data to suggest that loss of ECS results in symptoms of depression and anxiety [77]. Thus, deficiency of ECS can be induced by chronic stress and likely is a contributor to the negative consequences that follow.

4.1. Acute stress and ECS

There is considerable evidence that acute exposure of rodents and humans to stress results in changes in ECS. In rodent models, studies have focused primarily on changes in CNS ECS, while in humans, eCB concentrations in the circulation have been used as an index of ECS [78]. As described in section 2.4, acute stress exposure increases 2-AG and 2-AG/CB1R signaling in the hypothalamus, hippocampus, PFC and raphe.

On the other hand, acute stress exposure in rodents decreases amygdalar and PFC concentrations of AEA [68, 79–81]. The acute effect of stress to reduce AEA concentrations was accompanied by an increase in the activity of FAAH. The effect on AEA is not likely mediated by glucocorticoids, because exogenous glucocorticoids produce a rapid increase in tissue contents of AEA in the amygdala, hippocampus and hypothalamus [57] and in hypothalamic slices [58]. These changes are transient, returning to baseline even when the concentration of circulating CORT is still elevated. Interestingly, the increase in AEA was not accompanied by increases in two other NAEs, suggesting that reduction in activity of

FAAH is not responsible for the change. It is possible that AEA synthesis has increased, or that an AEA-selective catabolic process has been inhibited. Interesting recent data from Hill and colleagues indicate that CRH, through actions at CRH-R1 receptors, increases FAAH activity and decreases AEA in the amygdala [82]. These data, together with the rapid time course of change, indicate that a reduction in AEA is a very early event in the stress response and is not a glucocorticoid-mediated effect, at least in the amygdala.

The glucocorticoids also produce delayed effects that occur 1–5 hours after stress, particularly in the hippocampus, basolateral amygdala, medial PFC (mPFC) and ventral tegmental area of the midbrain [4]. The delayed effects of the glucocorticoids are hormone-like and are mediated by binding to GR and mineralocorticoid (MR) receptors [83]. GRs are ubiquitously distributed in brain, with very high densities in the PVN of the hypothalamus, hippocampal CA1 and dentate gyrus, lateral septum and the nucleus tractus solitarius (NTS) [83]. The brain distribution of MR is more restricted than GR and overlaps GR distribution in the hippocampal regions and the lateral septum [83]. The affinity of CORT for MR is ten times higher than for GR such that MR are occupied at basal concentrations of CORT [4]. An early study demonstrated that adrenalectomy resulted in increased CB1R mRNA expression in the caudate putamen of male rats [84], suggesting that glucocorticoids exert a negative effect on the transcription of CB1R. In support of this notion, prolonged exogenous CORT treatment reduces CB1R binding site density in the hippocampus [85, 86] and amygdala [86]. However, the changes in binding site density were not accompanied by changes in mRNA [86], so it is possible that genomic GR effects were not involved in the effect. Rats injected with CORT daily for 10 days also exhibit decreased CB1R protein expression and function in primary sensory neurons through a GR mechanism [87]. Similarly, intense activation of the HPA axis significantly reduces CB1R mRNA expression in the frontal cortex measured 7 days later [88]. Although the specific role of GR in these effects was not determined, several of these studies are consistent with glucocorticoids reducing CB1R expression through transcriptional regulation.

4.2 Chronic Stress Alters CB1R Expression

Chronic unpredictable (also called chronic mild or chronic variable) stress (CUS) paradigms expose rodents to a variety of stressors presented in a random manner [89]. CUS results in increased basal CORT secretion, hyperactive HPA axis responses to stress, anhedonia and anxiety-like behaviors [89]. In both intact and gonadectomized male rats, CUS exposure results in a 50% decrease in CB1R binding site density and protein in the hippocampus [73, 90]. Interestingly, CUS increases CB1R protein expression in both intact and gonadectomized female rats [90].

Chronic exposure to homotypic (i.e. the stressor stays the same) stress also results in increased anxiety-like behaviors, but is less likely to elevate basal CORT concentrations and to induce body weight loss than CUS [91]. The difference is that animals can habituate to some of the effects of repeated homotypic stress exposure while the unpredictable nature of the CUS model prevents this. Repeated exposure to restraint (21 days of 6 hrs restraint per day) reduces CB1R binding site density in the dentate gyrus of the hippocampus, although the changes are less robust than occur following CUS [92]. Chronic restraint stress impairs

eCB-mediated suppression of GABA signaling in the CA1 region of the hippocampus [93], which is in accord with a loss of CB1R binding sites and protein.

Therefore, chronic stress exposure reduces CB1R expression and ECS in the hippocampus. Decreased ECS mediated regulation of GABA release in the hippocampus could have important implications for spatial learning and flexibility, which are altered by chronic stress exposure [5].

In contrast to the effects seen in the hippocampus, CUS increases CB1R binding site density in the PFC of male rats [94]. Similarly, male mice exposed for 4 days to 2 hours per day of immobilization and acoustic stress exhibit a significant increase in CB1R protein expression in PFC [95]. Chronic treatment of rats with the tricyclic antidepressant, imipramine, reverses the up-regulation of CB1R binding in the mPFC produced by CUS [94]. These findings suggest that the increase in CB1R binding site density in mPFC is secondary to an effect of stress to alter monoamine signaling.

CUS has been shown to reduce CB1R binding site density in the ventral striatum in rats [94] and to produce functional down-regulation of eCB signaling in the nucleus accumbens in mice [96]. These changes in ECS likely contribute to the anhedonic effects of CUS [96].

Other paradigms that exert a chronic stress have also been shown to alter CB1R protein or mRNA expression. For example, social isolation results in a significant increase in CB1R mRNA throughout the cortical regions of adult male rats compared to those raised in groups [97]. However, autoradiographic analyses did not detect changes in CB1R binding in cortical regions in socially isolated male rats [98]. On the other hand, CB1R binding was decreased in the supraoptic hypothalamic nuclei and ventrolateral thalamus and increased in subregions of the caudate putamen in singly-housed compared to group-housed rats [98]. Repeated episodes of alcohol withdrawal, which produces anxiety in rodents [99], resulted in a significant reduction of CB1R mRNA in the amygdala of male rats [100].

4.3 Chronic Stress Regulates FAAH and MAGL Expression and Activities

FAAH is the primary catabolic enzyme for AEA in the brain and its inhibition increases AEA concentrations throughout the brain [101]. Among other things, increased AEA concentrations are associated with decreased anxiety [69] and dampened HPA axis activation [67, 68] as a result of CB1R activation. Five putative GR binding elements have been identified in the promoter region of the mouse FAAH gene [102], and *in vitro* studies demonstrate that non-liganded, GR represses FAAH expression [102]. Interestingly, the addition of a GR agonist did not increase the repression *in vitro*, suggesting that the GR does not need to be bound by ligand to repress FAAH although ligand binding is necessary for GR translocation to the nucleus *in vivo*. Isolation stress in male rats produces very significant reductions (greater than 50% in most regions) in FAAH mRNA expression in cortical regions and throughout the dorsal and ventral striatum [97]. Interestingly, AEA concentrations measured in adult male rats raised in isolation were significantly elevated in the pyriform cortex compared to group housed rats, but were unchanged in PFC, nucleus accumbens and hippocampus [98]. Thus, the comparison of mRNA and AEA content data indicates that the large reduction in FAAH mRNA does not always result in increased AEA

content, and suggests that isolation stress could reduce AEA synthesis as well as decrease its catabolism in a brain-region specific manner.

FAAH protein is reduced by 40% in dorsal root ganglion (DRG) cells from rats chronically treated with CORT [87], which supports the hypothesis that GR activation decreases FAAH expression.

CORT also appears to have effects on FAAH activity that are independent of changes in FAAH expression. For example, chronic administration of CORT to male rats does not alter mRNA expression but produces an increase in the V_{max} for FAAH in the amygdala and hippocampus [86]. AEA concentrations in these brain regions are significantly reduced by chronic CORT administration, consistent with enhanced FAAH-mediated hydrolysis. Similarly, repeated restraint in male mice increases the V_{max} for FAAH and decreases AEA concentrations in amygdala and mPFC [81]. This is a brain region selective effect of stress since repeated restraint decreases FAAH V_{max} and increases AEA content in ventral striatum [81]. A reciprocal relationship between AEA content and FAAH activity is seen consistently in rodents exposed to chronic stress, suggesting that chronic stress increases catabolism of AEA by FAAH, which could contribute to hypoactive CB1R signaling. The mechanism by which CORT and stress alter the V_{max} for FAAH is not known, but the lack of clear demonstration that mRNA is reduced suggests post translational modification of the enzyme has occurred.

The predominant effect of social isolation on MAGL mRNA content is to increase expression throughout the cortex and in selected regions of the striatum [97]. In spite of this change, 2-AG contents in the PFC and pyriform cortex of male rats reared in isolated are significantly increased [98]. Among the possible explanations for the discordance of these results is that an increase in MAGL activity is accompanied by an increase in 2-AG synthesis such that no net change occurs. In support of this concept, 10 days of one-hour restraint stress in male mice increases mPFC 2-AG content and increases the V_{max} for MAGL activity; on the other hand, amygdalar 2-AG is also increased on day 10, but MAGL activity is unchanged from control [81]. These results suggest that repeated restraint exposure could increase the synthesis of 2-AG in many brain regions while MAGL activity is altered in a brain-region dependent manner, perhaps as a compensatory process.

Repeated exposure of male mice to restraint also increases 2-AG content in the basolateral amygdala and enhances 2-AG mediated synaptic plasticity at inhibitory synapses in that region [103]. Repeated restraint produced a decrease in MAGL protein associated with membranes, where it would presumably have more influence on 2-AG concentrations at the CB1R [103]. Furthermore, chronic inhibition of MAGL mimicked and occluded the effects of repeated restraint, suggesting that reduced MAGL activity is a major mechanism for the increase in 2-AG signaling in this model.

5. ECS and Habituation to Stress

Stress-induced HPA axis activation and behavioral response repertoires to repeated and predictable exposure to the same stress (homotypic stress) can habituate. Stress habituation is stress-specific, dependent upon the time between stress exposures, and the intensity of the

initial stress [104]. The ability of an individual to habituate to the effects of stress is one of the factors that confers resilience to the negative consequences of stress [9].

Emerging evidence demonstrates that enhanced ECS contributes to the mechanism of habituation to stress. While chronic exposure to unpredictable and variable stressors reduces CB1R-mediated signaling (section 4), repeated exposure to a short period of restraint sensitizes 2-AG mobilization. In particular, a single exposure to restraint does not alter contents of 2-AG in the limbic forebrain, mPFC, amygdala, or cerebellum of male mice [79]. However, when mice are exposed to increasing numbers of restraint episodes, carried out at the same time of day and for the same duration, 2-AG contents are progressively increased in these brain regions immediately after the stress offset [79, 81]. Similar changes are seen in the amygdala of the male rat [105]. Since chronic treatment with CORT also increases 2-AG content in the amygdala [106], it is possible that CORT mediates this effect. Recent data suggest that the mechanism for this effect is inhibition of MAGL function, perhaps as a result of changes in the subcellular distribution of MAGL such that less of the enzyme is present at the plasma membrane [103]. In contrast to the increase in 2-AG seen with repeated stress in these cortical regions, 2-AG contents are reduced in ventral striatum with repeated restraint [81], suggesting a different mechanism and purpose for this change in the reward system.

There is evidence that enhanced ECS contributes to the habituation of behavioral responses and HPA axis activation to the stressor. In male mice, systemic injection of CB1R antagonist prevents habituation to behavioral activation [79] and anhedonia [107] in response to stress exposure. Inhibition of ECS in the amygdala prevents habituation to HPA axis activation by stress in rats [105] while increased 2-AG concentrations in the amygdala prevent behavioral and synaptic adaptations to repeated restraint exposure in mice [103]. CB1R activation is also required for the habituation of innate fear behaviors in mice to repeated homotypic stress [108–110]. Recent data suggest that the effect of chronic stress exposure to enhance ECS content could also contribute to negative consequences of stress habituation. In particular, chronic treatment with CRH produces an increase in anxiety that is antagonized by CB1R antagonist treatment [111].

Taken together, these data suggest that the plasticity that is afforded by the ECS provides an important mechanism by which the brain can habituate to repeated, reliable stress exposures. As the ability to habituate to a non-threatening stimulus will allow an individual to conserve resources and avoid the consequences of chronic stress, this role of ECS could be one of the most important in the context of human psychiatric pathology.

6. ECS and Sympathetic Nervous System Responses to Stress

Exposure to an acute stress or anticipation of danger evoke characteristic physiological changes through activation of the neuronal defense pathway. Stress information is provided to the NTS from the amygdala, infralimbic cortex and the PVN [112]. Excitatory projections from the NTS to the locus coeruleus and ventromedial medulla activate preganglionic sympathetic neurons while inhibitory projections to the dorsal motor nucleus of the vagus and nucleus ambiguous inhibit the parasympathetic nervous system. There are several sites

in the CNS where ECS has been found to regulate activation of the SNS; interestingly, enhanced ECS can both increase and decrease SNS responses to stress.

Mobilization of ECS in the rat dorsal periaqueductal gray (PAG) enhances SNS activation [113, 114]. CB1R are found in the PAG [115] and microinjection of AEA into the dorsal PAG increases renal sympathetic nerve activity within 30 sec of injection [113]. These effects are inhibited by CB1R antagonist pretreatment and are consistent with CB1R-mediated inhibition of GABA release [114]. Microinjection of CB1R antagonist into the dorsal PAG also inhibits SNS responses evoked by hypothalamic stimulation [113]. As acute stress results in a rapid increase in the PAG tissue contents of both AEA and 2-AG [116], these results suggest that ECS in the dorsal PAG enhances or even enables stress-induced SNS activation.

On the other hand, the direct effects of CRH to increase SNS activation are inhibited by i.c.v. administration of CB1R agonists and increased by CB1R antagonists [117], suggesting a stress-inhibitory role for ECS. In support of this hypothesis, injection of AEA directly into the NTS of rats prolongs baroreceptor-induced sympathoinhibition [118]. These apparently contradictory effects of CB1R activity on SNS outflow illustrate the point that ECS is a local process, so it is not surprising that it exerts opposite effects on a circuit.

CB1R are expressed on terminals of sympathetic axons innervating blood vessels and there is evidence their activation inhibits the release of norepinephrine [20, 119]. CB1R mRNA has been detected in superior cervical ganglion [20], which is consistent with CB1R protein expression by post-ganglionic, sympathetic neurons. Mice lacking CB1R selectively in sympathetic neurons are lean and resistant to diet-induced obesity, which the authors hypothesize is due to the loss of CB1R-mediated suppression of norepinephrine release, which results in increased lipid oxidation and thermogenesis as a result of increased sympathetic tone [120]. CB1R on sympathetic terminals in bone inhibit norepinephrine release and oppose the effects of the SNS to reduce bone formation [121, 122]. CB1R activation suppresses norepinephrine release evoked by perivascular nerve stimulation in the isolated heart [123]. The source of eCB that provides innervation of these receptors is not known, although the retrograde signaling paradigm of ECS in brain suggests that the tissues receiving the neuronal input are a possible source. The inflammatory molecule, lipopolysaccharide (LPS), is able to mobilize functional ECS at sympathetic terminals, suggesting recruitment of the CB1R during inflammation. It is possible that ECS at the sympathetic terminal functions as a local feedback modulator to protect tissues from excessive SNS activation.

An important component of the sympathetic response to stress is a coordinated effect on the cardiovascular system. Activation of β-adrenergic receptors of the heart and blood vessels by norepinephrine released from sympathetic terminals results in alterations in heart rate, contractile force and blood flow to muscles and skin that are necessary to support the fight or flight response. It is well accepted that chronic stress increases the risk of cardiovascular diseases, particularly those of the heart [124]. CB1R are expressed by non-neuronal cells of the cardiovascular system, including cardiomyocytes, vascular smooth muscle cells and endothelium [24, 125, 126]. While the majority of available evidence indicates that

endogenous CB1R signaling does not contribute to the regulation of cardiovascular function under normal conditions, it is likely that CB1R signaling in this system is recruited under various pathophysiological states, including inflammation [127] and profound hypotension [128]. O'Sullivan and colleagues recently reviewed the roles for the broadly considered endocannabinoid system in the effects of stress on the cardiovascular system and concluded that there are multiple possible sites of interaction at all levels, including the brain, sympathetic nerves, HPA axis and end organs [124].

7. CB1R and Immune Responses to Stress

Among the many effects of stress is a profound ability to suppress the immune system [129]. This is the result of both arms of the stress response. Glucocorticoids act through GR receptors to alter the expression of a variety of cytokines and inflammatory mediators, particularly TNFα [130]. Postganglionic fibers of the SNS innervate the spleen and release norepinephrine in response to activation, which reduces inflammatory cytokine production through β-adrenoreceptor activation [131]. In light of the data discussed in section 6 that ECS inhibits release of norepinephrine from sympathetic terminals, a logical hypothesis is that ECS, through CB1R activation, promotes stress-induced inflammation and that CB1R antagonists will be anti-inflammatory. There is evidence to support this hypothesis. In a study of the anti-inflammatory mechanism of CB1R antagonists, Mnich and colleagues demonstrated that the site of action of this effect was at the sympathetic terminals in the spleen [132]. Their data strongly suggest that, at least in the inflamed state, eCBs mobilized in the spleen function to inhibit norepinephrine release and thus, decrease the anti-inflammatory influence of SNS activation.

Periodontitis includes inflammation of the gums and other tissues supporting the teeth [133]. Rettori and colleagues reported that gingival injection of AEA in rats with periodontitis also exposed to restraint stress reduced the elevation of circulating CORT, and reduced gingival tissue necrosis factor alpha (TNFα) and IL-1β immunoreactivities [134]. These effects were reversed by combined CB antagonist treatment [134]. Thus, general activation of ECS in the inflamed area of the tooth exerts an immune-suppressive effect that extends to a reduction in basal HPA axis activity.

8. ECS, Stress and the Gastrointestinal Tract

All components of ECS are found in the gastrointestinal (GI) tract. CB1R have been shown to be present in cholinergic neurons in both the myenteric and submucosal plexi of the ENS [135, 136].

Stress affects multiple functions of the GI tract, including gastric secretion, motility, epithelial permeability and barrier function, and mucosal blood flow [137]. Acute and chronic stress are associated with lower pain thresholds and visceral hypersensitivity to painful stimuli [138]. Male rats exposed to water avoidance stress exhibit GR-mediated decreases in CB1R expression in DRG neurons, a change that is hypothesized to contribute to stress-induced hypersensitivity to colorectal distension [87]. On the other hand, four days of exposure of male rats to partial restraint increases CB1R expression in the colon [139].

This stress protocol also produces visceral hypersensitivity, and the authors suggest that increased colonic CB1R expression is an attempt to normalize pain sensitivity [139].

$CB1R^{-/-}$ mice exposed to 4 days of 2-hour immobilization and acoustic stress exposure exhibit increased permeability of the colonic barrier; enhanced inflammation; lower IgA secretion and higher bacterial translocation into the mesenteric lymph nodes than wild type mice [140]. IgA secretion by the GI tract is the first line defense against pathogens, through its ability to neutralize viruses, bind toxins and food antigens, and to reduce bacterial binding to epithelial cells [141]. Thus, the loss of IgA secretion, together with loss of the intestinal barrier function and reduced visceral hypersensitivity to pain, suggest an important homeostatic role of CB1R in the intestine.

9. Conclusions

Overwhelming data support the hypothesis that the ECS is a critical component of homeostatic regulation of the body. Endocannabinoid/CB1R signaling is primarily stress-inhibitory, reducing both the endocrine and neuronal responses to stress. CB1R signaling participates in habituation to stress exposure, which is a protective mechanism designed to dampen responses to a non-threatening stimulus. On the other hand, chronic stress exposure decreases endocannabinoid/CB1R signaling. Given the vital role of CB1R activation in maintaining hedonia and reducing anxiety, the reduction of CB1R signaling is hypothesized to contribute to the negative consequences of stress.

There is some support for the "Endocannabinoid Deficiency" hypothesis in humans. Recent data that proteins of the ECS exhibit polymorphisms in humans suggests that differences in the tone of ECS could contribute to vulnerability or resilience to psychopathology [142]. A very interesting recent study found that CB1R genotype exerts a significant effect on the likelihood that early childhood neglect will result in anhedonia in adulthood [143] and animal studies strongly suggest that early life stress alters ECS [144]. Thus, alterations of ECS could be an important link between early life stress and psychopathology in later life. Finally, emerging data indicate that significant sex differences in the role of ECS in the regulation of stress responsivity [145]. It is also generally acknowledged that most psychiatric disorders exhibit clear sex differences, with substance abuse disorders, antisocial personality and attention deficit disorder being more common in men; while depression, anxiety and eating disorders are more common in women [146].

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Abbreviations

2-AG	2-arachidonoylglycerol
AEA	*N*-arachidonylethanolamine
CB1R	type 1 cannabinoid receptor

CORT	corticosterone
COX2	cyclooxygenase type 2
CRH	corticotropin releasing hormone
CUS	chronic, unpredictable stress
DAG	diacylglycerol
DAGL	diacylglycerol lipase
DRG	dorsal root ganglion
eCBs	endocannabinoids
ECS	endocannabinoid signaling
FAAH	fatty acid amide hydrolase
GR	glucocorticoid receptor
HPA	hypothalamic-pituitary-adrenal
MAGL	monoacylglycerol lipase
mPFC	medial prefrontal cortex
MR	mineralocorticoid receptor
NAE	*N*-acylethanolamine
NAPE	*N*-acyl-phosphatidylethanolamine
NTS	nucleus tractus solitarius
PAG	periaqueductal gray
PFC	prefrontal cortex
PLC	phospholipase C
PVN	paraventricular nucleus
SNS	sympathetic nervous system
THC	Δ^9-tetrahydrocannabinol

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Highlights

- CB1 receptor signaling is mobilized by acute stress and serves to dampen the endocrine responses
- Chronic variable stress down-regulates CB1 receptor signaling in the hippocampus and ventral striatum
- Increased CB1 receptor signaling contributes to habituation of endocrine and some behavioral responses to repeated stress
- Reduced CB1 receptor signaling exacerbates the effects of stress and may contribute to its negative consequences