Published in final edited form as:

Neuropharmacology. 2011 September ; 61(3): 414–420. doi:10.1016/j.neuropharm.2011.02.016.

Modulation of the Serotonin System by Endocannabinoid Signaling

Samir Haj-Dahmane and Roh-Yu Shen

Research Institute on Addictions, University at Buffalo, State University of New York, 1021 Main Street, Buffalo, New York 14203

Abstract

The cannabinoid CB₁ receptors and their endogenous agonists, endocannabinoids (eCBs), are ubiquitously distributed throughout the central nervous system (CNS), where they play a key role in the regulation of neuronal excitability. As such, CB signaling has been implicated in the regulation of a myriad of physiological functions ranging from feeding homeostasis to emotional and motivational processes. Ample evidence from behavioral studies also suggests that eCBs are important regulators of stress responses and a deficit in eCB signaling contributes to stress-related disorders such as anxiety and depression. The eCB-induced modulation of stress-related behaviors appears to be mediated, at least in part, through the regulation of the serotonergic system. In this article, we review the role of eCB signaling in the regulation of the serotonergic system with special emphasis on the cellular mechanisms by which cannabinoid CB₁ receptors modulate the excitability of dorsal raphe serotonin neurons.

Keywords

Endocannabinoid; serotonin; dorsal raphe; stress; synaptic transmission

1. Introduction

The endocannabinoid (eCB) system, which is composed of cannabinoid receptors, the molecular target of Δ^9 -tetrahydrocannabinol (Δ^9 -THC) found in cannabis, and their endogenous agonists including N-arachidonylethanolamine (anandamide/AEA) and 2-arachidonoylglycerol (2-AG) plays an important role in the regulation of stress-related behaviors (Viveros et al., 2005). In humans, the mood elevating and euphoric effects caused by the recreational use of cannabis are associated with reduced stress and anxiety (Tournier et al., 2003). In contrast, chronic cannabis abuse can lead to increased anxiety and panic attacks (Hall and Solowji, 1998; Tournier et al., 2003). Similarly, the results of animal studies show that pharmacological blockade or genetic deletion of CB₁ receptors increases stress-related behaviors (Arevalo et al., 2001; Haller et al., 2004a) and impairs the extinction of conditioned fear-responses (Marsicano et al., 2002). On the other hand, pharmacological enhancement of eCB signaling via the blockade of eCB metabolism and/or uptake reduces stress-related behaviors and facilitates the extinction of conditioned fear-responses (Kathuria

Corresponding author: Samir Haj-Dahmane, Ph.D, Research Institute on Addictions, University at Buffalo, 1021 Main Street, Buffalo, NY 14203., Phone: (716) 887-2231, Fax: (716) 887-2477, dahmane@ria.buffalo.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

et al., 2003). Based on these observations, eCB signaling has been targeted for the development of novel pharmacotherapies of anxiety and stress-related mood disorders.

Results from behavioral studies have suggested that many behavioral effects of eCB signaling including the regulation of stress responses are, at least in part, mediated by the modulation of the serotonin (5-HT) system (Griebel et al., 2005; Marco et al., 2004). Therefore, several recent studies have investigated the cellular/molecular mechanisms by which eCB signaling modulates the function of the 5-HT system. The novel and exciting results of these studies prompted the current review. In this article, we will first provide background review on eCB signaling and its neurophysiological effects in the CNS. We will then describe the recent development regarding the regulation of 5-HT system by eCB signaling with the emphasis on the cellular/molecular mechanisms by which eCBs control the excitability of dorsal raphe (DR) 5-HT neurons.

2. Endocannabinoid signaling in the mammalian brain

Endocannabinoids are lipid signaling molecules with potent actions at cannabinoid receptors. So far, two cannabinoid receptors (CB1 and CB2) have been cloned and characterized pharmacologically. The CB1 receptors are expressed at high density throughout the CNS (Herkenham et al., 1991; Matsuda et al., 1993; Egertová and Elphick, 2000) and at lower density in peripheral tissues and immune cells (Galiegue et al., 1995). CB2 receptors are initially thought to be predominantly expressed in the peripheral tissues and immune cells (Munro et al., 1993), but results from recent studies have reported a limited neuronal and microglial expression of these receptors in the CNS (Van Sickle et al., 2005). The CB1 and CB2 receptors belong to the G-protein coupled receptor (GPCR) family and signal through $G_{o/i}$ family of G-proteins (Devane et al., 1988; Matsuda et al., 1990). Activation of both of these receptors inhibits the adenylyl cyclase (Bayewitch et al., 1995) and stimulates mitogen-activated protein kinases (Bouaboula et al., 1995). Stimulation of CB1 receptors also inhibits N and P/Q type of voltage-gated calcium channels (Mackie and Hille, 1992) and activates G-protein-coupled inward rectifier potassium (GIRK) current (Mackie et al., 1995).

Endocannabinoids have also been shown to activate the transient receptor potential (TRP) superfamily of cation-non selective channels, which are involved in the transduction of remarkable diverse stimuli, including temperature, pH, mechanical and osmotic stimuli, taste, the effects of xenobiotic substances, and endogenous lipids (Venkatachalam and Montel, 2007). Among these channels, TRPV1, also known as vanilloid receptor 1 (VR1) is the first TRP channel cloned. This channel was first identified as a receptor for capsaicin, a pungent ingredient of hot chili pepper (Caterina et al., 1997). Subsequent work from different research groups has established that eCBs such as anandamide and N-arachidonyl dopamine, but not 2-AG, bind with high affinity to TRPV1 and activate these channels (Zygmunt et al., 1999, for review, see Tóth et al., 2009). More recently, results from several electrophysiological studies have provided strong evidence that the activation of TRPV1 by eCBs modulates synaptic transmission and plasticity in various brain areas (Grueter et al., 2010; Chávez et al., 2010). Together results from these studies suggest that TRPV1 channel may represent an “ionotropic” cannabinoid receptor.

In addition to classical G-protein-coupled membrane receptors and TRPV1 channels, eCBs have also been shown to activate peroxisome proliferator-activated receptors (PPARs) (Sun et al., 2007), which are ligand-activated transcription factors and belong to the family of nuclear receptors family (Michalik et al., 2006). Activated PPARs form heterodimers with retinoid X receptors to control gene transcription. Many of the PPARs target genes are enzymes associated with lipid turnover such as CoA dehydrogenase and lipoprotein lipase.

As such, in the peripheral tissues, such as liver and adipose tissues, activation of the PPAR pathways has been shown to play a critical role in the regulation of adipogenesis and glucose homeostasis (Tontonoz et al., 1994; Chinetti et al., 2000). In the CNS, PPARs are involved in the regulation of inflammatory responses (Lahrke and Lazar, 2005) and neurogenesis (Qi et al., 2010). Activation of the PPAR pathway has been shown to reduce the inflammatory responses, decrease the oxidative stress, inhibit apoptosis induced by traumatic brain injury, and promotes neurogenesis (Qi et al., 2010). These observations suggest that the activation of PPARs by eCBs may play a neuroprotective role during oxidative stress or in response to traumatic brain injuries.

The two main eCBs are anandamide (Devane et al., 1992) and 2-AG (Mechoulam et al., 1995). It is now generally accepted that, unlike classical neurotransmitters and neuropeptides, AEA and 2-AG are not constitutively synthesized and stored in synaptic vesicles for future release. Instead, they are synthesized “on demand” from postsynaptic membrane phospholipid precursors via activity-dependent stimulation of specific phospholipase enzymes (Di Marzo et al., 1994; Stella et al., 1997). The activity-driven eCB synthesis and release requires an increase in intracellular calcium (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson et al., 2001). The major pathway for the biosynthesis of anandamide is via the precursor N-arachidonoyl phosphatidylethanolamine (NAPE), which is generated by a calcium-dependent activation of N-acyltransferase. Anandamide is then produced by the hydrolysis of NAPE, which is catalyzed by phospholipase D (Di Marzo et al., 1994; Sugiura et al., 1996). On the other hand, 2-AG is synthesized by the hydrolysis of phosphatidyl inositol biphosphate (PIP2) via phospholipase C (PLC), which leads to the formation of the second messengers inositol-(1, 4, 5)-triphosphate (IP₃) and 1, 2 diacylglycerol (DAG). The latter is then converted to 2-AG by diacylglycerol lipase (DAGL) (Stella et al., 1997). Two closely related genes encoding DAGL, DAGL- α and DAGL- β have been cloned (Bisogno et al., 2003). Recent studies using mice lacking either DAGL- α or DAGL- β have reported an 80 % reduction in 2-AG levels in the brain and spinal cord of DAGL- $\alpha^{-/-}$ mice and 50 % reduction in the brain of DAGL- $\beta^{-/-}$ mice. These results suggest that DAGL- α plays a major role in the biosynthesis of 2-AG in the CNS (Tanimura et al., 2010).

The eCB signaling is terminated via a two-step process involving an active uptake of eCB by a membrane transporter, which remains to be characterized, and a subsequent intracellular hydrolysis (Beltramo et al., 1997; Hillard et al., 1997). Anandamide is metabolized by fatty acid amid hydrolase (FAAH) (Cravatt et al., 1996), whereas 2-AG is metabolized by monoglyceride lipase (MGL) (Goparaju et al., 1999). 2-AG can also be hydrolyzed by the recently described serine hydrolase α - β -hydrolase domain 6 (ABHD6) (Marrs et al., 2010), which is expressed in the brain. Genetic deletion of ABHD6 has been shown to increase the accumulation and efficacy of 2-AG in the brain (Marrs et al., 2010). In addition to these catabolic enzymes, both anandamide and 2-AG can be metabolized by cyclooxygenase type 2 (COX 2) into prostaglandins (Kozak et al., 2000). For a detailed review of the multiple routes of eCB metabolism see Di Marzo, 2008.

3. Neurophysiology of eCB signaling in the CNS

In the CNS, the effects of eCBs are largely mediated by CB1 receptors. The predominant localization, although not exclusive of these receptors on presynaptic axon terminals, suggests a key role of eCBs in controlling synaptic transmission (Egertová and Elphick, 2000; Tsou et al., 1998). Consistent with this notion, it is now well established that eCBs inhibit GABA (Szabo et al., 1998; Wilson et al., 2001) and glutamate-mediated synaptic transmission (Auclair et al., 2000; Azad et al., 2003) by suppressing neurotransmitter release. In addition to the modulation of neurotransmitter release, in few brain areas, eCBs

have also been shown to exert a direct postsynaptic effect on neuronal excitability. For instance, in the cerebellum and cerebral cortex, eCBs hyperpolarize and suppress the firing activity of inhibitory interneurons (Kreitzer and Regehr, 2001). This inhibitory effect is mediated by postsynaptic CB1 receptor-induced activation of inward rectifier potassium current (Bacci et al., 2004).

The reports of the expression of CB2 receptors in the CNS, albeit limited (Van Sickle et al., 2005), suggests that these receptors may mediate some of the neurophysiological effects of eCBs. Consistent with this notion, activation of CB2 receptors has been shown to inhibit glutamate release in the entorhinal area of the cerebral cortex (Morgan et al., 2009). Additional studies, however, are required to elucidate the detailed mechanisms underlying the neurophysiological effects of CB2 receptors.

Results from eCB research in the last decade have firmly established that, in the CNS, eCBs are the major retrograde signaling molecules that fine tune synaptic transmission and mediate several forms of short-term and long-term synaptic plasticity (for review see, Kano et al., 2009). A brief postsynaptic membrane depolarization has been shown to trigger the synthesis and release of eCBs, which in turn act retrogradely at presynaptic CB1 receptors to induce a short-term suppression of neurotransmitter release at both the excitatory (Kreitzer and Regehr, 2001) and inhibitory synapses (Ohno-Shosaku et al., 2001; Wilson et al., 2001). The eCB-mediated short-term synaptic depression observed at glutamate and GABA synapses is called depolarization-induced suppression of excitation (DSE) and inhibition (DSI), respectively. Retrograde eCB signaling has also been shown to mediate activity-dependent long-term depression (LTD) of glutamatergic (Gerdeman et al., 2002; Haj-Dahmane and Shen, 2010) and GABAergic synaptic transmission (Chevalleyre and Castillo, 2003; Marsicano et al., 2002) throughout the brain. The eCB-mediated LTD is mainly caused by a persistent decrease in neurotransmitter release and represents one of the best examples of presynaptic forms of long-term synaptic plasticity (for review see, Heifets and Castillo, 2009). Taken together, these studies indicate that an important physiological role of the retrograde eCB signaling is to provide a mechanism by which neurons can auto-regulate and fine tune the strength of their synaptic inputs.

4. Modulation of the stress responses by eCB signaling and the 5-HT system

The notion that the eCB system may be involved in the regulation of the stress responses is rooted in the observation that the recreational use of cannabis in humans has profound effects on mood and stress-related behaviors (Williamson and Evans, 2000). Subsequent animal studies using different stress models have confirmed that eCB signaling play a critical role in the regulation of stress-related behaviors, and neuronal circuitries mediating stress. For example, results from *in vivo* studies show that administration of glucocorticoids or the activation of HPA axis in response to various stressors such as, restraint stress enhances eCB levels in brain areas known to mediate the neurobehavioral effects of stress (Hill et al., 2007, 2009). Similarly, within the periventricular nucleus of the hypothalamus (PVN), *in vitro* administration of glucocorticoids evokes a rapid induction of eCB synthesis and release (Di et al., 2003). The rapid responses to glucocorticoids are mediated by non-genomic mechanism involving putative G-protein coupled membrane receptors (Di et al., 2003). The finding that stress and glucocorticoids engage the eCB system suggests that eCB signaling may contribute to the regulation of the neuroendocrine responses to stressors. Consistent with this notion, pharmacological blockade of CB1 receptor has been shown to enhance stress-induced activation of the HPA axis and glucocorticoid secretion (Patel et al., 2004). Similarly, genetic deletion of CB1 receptor profoundly enhances the secretion of stress hormones (i.e. ACTH and corticosterone) elicited by an array of stressors (Uriguen et

al., 2004; Steiner et al., 2008). In addition, behavioral studies using CB1 knock out mice or pharmacological blockers of CB1 receptor have shown an increase in stress-related behaviors (Haller et al., 2004a; 2004b). In contrast, pharmacological enhancement of eCB signaling either via the blockade of eCB hydrolysis and/or uptake has been shown to reduce the behavioral responses to stress (Kathuria et al., 2003). Collectively, these studies lead to the current notion that stress engages the eCB system to constrain further activation of the HPA axis. As such, a disruption of eCB signaling may lead to excessive activation of the HPA axis and heightened sensitivity to stress, which are the major features of mood and anxiety disorders.

It is now well documented that the activity of the HPA axis is also under the control of the 5-HT system. Indeed, several studies have shown that 5-HT through the activation of 5-HT receptors located in the PVN regulates neuroendocrine responses to stress (for review see, Lanfumey et al 2008). For instance, activation of the 5-HT_{1A} receptors has been shown to reduce the secretion of ACTH and corticosterone induced by an array of stressors (Carrasco and Van de Kar, 2003). In addition, results from behavioral studies have shown that activation of the 5-HT_{1A} receptors induces anxiolytic-like behaviors in various animal models. In contrast, pharmacological blockade or genetic disruption of the 5-HT_{1A} receptors, generally, augments stress responses and increases anxiety-like behaviors (Gingrich and Hen, 2001). The involvement of the 5-HT system in the regulation of the neurobehavioral responses to stress is further demonstrated by the fact that 5-HT_{1A} receptor agonists and the selective serotonin uptake inhibitors (SSRIs) are clinically used to treat anxiety and stress-related mood disorders (Nemeroff, 2003).

Because of the prominent and converged roles played by the eCB and 5-HT systems in the regulation of the behavioral responses to stress, several recent studies have begun to examine the functional interaction between these two systems and its role in the regulation of stress responses. In General, results of these studies show that the 5-HT system participates in the eCB-induced modulation of the HPA axis and stress responses. For instance, eCBs have been shown to attenuate the response of the HPA axis to acute restraint stress by facilitating 5-HT mediated neurotransmission (Resstel et al., 2009). Furthermore, the anxiolytic-like behavioral effects induced by an increase in eCB tone appears to be mediated by an alteration of 5-HT_{1A} and 5-HT_{2A} receptors mediated neurotransmission (Bambico et al., 2010). Together, these studies support the idea that the eCB signaling modulates the behavioral responses to stress, at least, in part, via the regulation of the 5-HT system, which will be discussed in the section below.

5. Regulation of the 5-HT system by eCB signaling

Early evidence for a role of eCB signaling in the regulation of the 5-HT system is suggested by behavioral studies showing a high level of functional overlap between the 5-HT and eCB systems. For example, both 5-HT and eCB systems regulate body temperature (Malone and Taylor, 2001), feeding behavior (Ward et al., 2008), sleep and arousal (Murillo-Rodriguez et al., 2008), and emotional processes (Marco et al, 2004). More importantly, the eCB-mediated modulation of these physiological functions requires the participation of the 5-HT system (Malone and Taylor, 2001; Marco et al., 2004). Many neurochemical studies have since directly examined how eCB signaling modulates the function of the 5-HT system. The results of these studies show that the eCB signaling influences 5-HT release. Indeed, *in vivo* activation of the CB1 receptors has been shown to inhibit 5-HT release in the projection areas of DR 5-HT neurons such as the prefrontal cortex and hippocampus (Egashira et al., 2002). Similarly, in cortical slice preparation, stimulation of these receptors reduces the electrically and calcium-induced 5-HT release (Nakazi et al., 2000). In contrast, pharmacological blockade of CB1 receptors has been shown to enhance the basal

extracellular levels of 5-HT in the medial prefrontal cortex (Aso et al., 2009; Darmani et al., 2003; Tzavara et al., 2003). Taken together, the results of these studies indicate that eCBs reduce serotonin release in the CNS via the activation of CB1 receptors.

In addition to the modulation of serotonin release, eCB signaling has also been shown to control the function and expression of various 5-HT receptors in the CNS. For instance, results from recent studies in CB1 knock out mice show that genetic deletion of CB1 receptors profoundly reduces the functional coupling of 5-HT_{1A} and 5-HT_{2A} receptors as measured by the ability of these receptors to stimulate [³⁵S] GTPγS binding in the hippocampus and prefrontal cortex. The reduction in the function of 5-HT_{1A} and 5-HT_{2A} receptors appears to be associated with no significant changes in their expression levels (Aso et al., 2009; Mato et al., 2007). Genetic deletion of CB1 receptors also reduces the ability of the somatodendritic 5-HT_{1A} receptors located in the DR to inhibit the firing activity of 5-HT neurons (Aso et al., 2009). The impact of eCB signaling on the function of 5-HT receptors was also examined after chronic activation of CB1 receptors with either Δ⁹-THC or synthetic CB1 receptor agonists. The results of these studies show an increased expression and function of 5-HT_{1A} receptors in the hippocampus (Moranta et al., 2009; Zavitsanou et al., 2010). Together these studies provide strong evidence that eCBs via activation of central CB1 receptors modulate the release of 5-HT as well as the function of 5-HT receptors. Such a regulatory control may represent a mechanism that underlies the functional cross talk between the eCB and 5-HT systems.

6. Modulation of the excitability of DR 5-HT neurons by eCB signaling

Results from early anatomical studies have shown that both CB1 receptors and the enzymes involved in the synthesis and catabolism of eCBs are expressed in the DR (Egertova et al., 2003; Matsuda et al., 1993; Tsou et al., 1998), suggesting that eCB signaling may play an important role in the regulation of DR 5-HT neuron function. Consistent with this notion, *in vivo* and *in vitro* electrophysiological studies have reported that eCBs as well as exogenous cannabinoids regulate the excitability of DR 5-HT neurons (Bambico et al., 2007; Gobbi et al., 2005; Haj-Dahmane and Shen 2005; 2009). For example, results from an *in vivo* extracellular recording study show that systemic administration of FAAH inhibitors, which presumably increases anandamide levels in the CNS, enhances the firing activity of DR 5-HT neurons via the activation of CB1 receptors (Gobbi et al., 2005). Similarly, *in vivo* administration of the selective CB1 receptor agonist Win 55,212-2 has been shown to increase the firing rate of DR 5-HT neurons (Bambico et al., 2007). The eCB-induced excitation of DR 5-HT neurons reported *in vivo* is blocked after lesion of the prefrontal cortex, suggesting that it is an indirect effect mediated by activation of CB1 receptors presumably located in the prefrontal cortex (Bambico et al., 2007).

Evidence for a direct role of eCB signaling and CB1 receptors located in the DR in regulating the excitability of 5-HT neurons comes from *in vitro* intracellular electrophysiological studies (Haj-Dahmane and Shen, 2005; 2009). Results from these studies show that, while eCBs as well as synthetic CB1 receptor agonists have no significant effects on the intrinsic excitability of DR 5-HT neurons, they profoundly reduce the strength of glutamate synapses impinging on these neurons. Specifically, administration of anandamide strongly reduces the amplitude of glutamate-mediated excitatory postsynaptic currents (EPSCs). Similarly, activation of CB1 receptors with WIN 55,212-2 reduces glutamatergic synaptic transmission to DR 5-HT neurons. The inhibitory effect of eCBs as well as the synthetic CB1 receptor agonists is readily blocked by the selective CB1 receptor antagonist AM 251, indicating that the eCB-induced modulation of glutamatergic synaptic transmission in DR 5-HT neurons is mediated by activation of CB1 receptors. The results of these studies also show that the CB1 receptor-induced suppression of glutamatergic

transmission is consistently associated with a significant decrease in the probability of glutamate release. Such finding indicates that the inhibition of glutamatergic synaptic transmission to DR 5-HT neurons is mainly caused by a presynaptic suppression of glutamate release. The outcome of these studies also suggests that CB1 receptors are most likely located on glutamatergic terminals impinging on DR 5-HT neurons. In addition to the inhibition of glutamate release, a recent *in vitro* extracellular recording study reports that eCB tone in the DR can regulate the firing activity of 5-HT neurons by regulating GABAergic transmission (Mendiguren and Pineda, 2009). However, the detailed cellular mechanism of such regulation remains to be determined.

The general view that emerges from the above studies is that eCBs regulate the activity of DR 5-HT neurons primarily, if not exclusively, via the regulation of their excitatory and inhibitory inputs (see, Fig. 1). By modulating the function of both the excitatory and inhibitory synapses, it is possible that eCBs exert a bidirectional modulation of the activity of DR 5-HT neurons. The net effect of eCBs on the firing activity of DR 5-HT neurons will largely depend on the synaptic strength and CB1 receptor density on the axon terminals of each of the inputs impinging on DR 5-HT neurons. Clearly, additional anatomical studies are required to determine the colocalization of these receptors with GABA and glutamate and their density on synaptic terminals impinging on DR 5-HT neurons. The results of these studies are necessary to formulate a comprehensive model of eCB modulation of 5-HT neurotransmission.

7. The “on demand” release of eCBs from DR 5-HT neurons control synaptic transmission

A major finding from our electrophysiological study is that DR 5-HT neurons synthesize and release eCBs in an activity-dependent manner (Haj-Dahmane and Shen, 2005; 2009). The activity driven-eCB synthesis and release from DR 5-HT neurons can be initiated by postsynaptic membrane depolarization and the subsequent increase in intracellular calcium or in response to the activation of postsynaptic G-protein coupled receptors. Indeed, a brief membrane depolarization of DR 5-HT neurons has been shown to induce DSE. The DSE requires an increase in postsynaptic intracellular calcium, and is caused by a presynaptic decrease in glutamate release. More importantly, like in other brain areas, the DSE in DR 5-HT neurons is readily blocked by CB1 receptor antagonists, confirming that the calcium-driven eCB synthesis and release from DR 5-HT activates presynaptic CB1 receptors and mediates the DSE at glutamate synapses impinging on DR 5-HT neurons (Haj-Dahmane and Shen, 2009).

The synthesis and release of eCBs from DR 5-HT neurons can also be triggered by the activation of postsynaptic G-protein coupled receptors, most notably the orexin receptors (OXR), which signal through the $G_{\alpha_{q/11}}$ family of G-proteins (Haj-Dahmane and Shen, 2005). Activation of these receptors with orexin B, also called hypocretin 2, increases the excitability of DR 5-HT neurons (Liu et al., 2002) and induces a profound inhibition of glutamatergic synaptic transmission to DR 5-HT neurons (Haj-Dahmane and Shen, 2005). The orexin-induced depression of synaptic transmission is mediated by the activation of postsynaptic OXR and caused by presynaptic decrease in glutamate release, indicating the involvement of retrograde messengers. Examination of the retrograde messengers involved reveals that the OXR-induced suppression of glutamate release is blocked by the CB1 receptor antagonist AM251 and occluded by the CB1 receptor activation. Such findings demonstrate that the OXR-induced inhibition of glutamatergic synaptic transmission is mediated by retrograde eCB signaling (Haj-Dahmane and Shen, 2005). The results of this study also show that the OXR-driven eCB synthesis and release involves the activation of the phospholipase C and DAG lipase pathway, suggesting that 2-AG synthesized by DR 5-

HT neurons is most likely the retrograde messenger that controls glutamatergic synaptic transmission in DR 5-HT neurons. However, additional studies are required to test the involvement of anandamide and others eCB species.

It is interesting to note that, in other brain areas, the activation of other $G_{q/11}$ coupled receptors such as group I metabotropic glutamate receptors (mGluR1), M_1 muscarinic receptors and the serotonin 5-HT_{2A} receptors also triggers the synthesis and release of eCBs (Best and Regehr, 2008; Maejima et al., 2001), which mediate retrograde control of synaptic transmission. Together, these studies support the notion that eCB synthesis and release induced by the activation of $G_{q/11}$ coupled receptor represents a widespread mechanism that mediate retrograde modulation of synaptic transmission in the CNS. In this context, it is tempting to speculate that other $G_{q/11}$ coupled receptors located in the DR may also stimulate eCB synthesis. Among these receptors are the α_1 -adrenergic receptors, which are highly expressed in the DR and play a central role in maintaining the pacemaker firing activity of DR 5-HT neurons recorded *in vivo* and *in vitro* (Brown et al., 2002). The α_1 receptors are activated by the noradrenergic input from the locus coeruleus, which is an important brain area mediating the stress responses. Therefore, the α_1 receptors located on DR 5-HT neurons might represent another convergence point for the regulation of stress by eCBs and 5-HT system. Future studies are required to investigate the role of these receptors in the regulation of eCB signaling in DR 5-HT neurons.

8. Concluding remarks

Results from the above studies indicate that eCBs through activation of CB1 receptors provide an important regulatory control of 5-HT system functions. This regulatory control occurs at the level of DR 5-HT neuron cell body as well as at their projection areas. In the projection areas, eCB signaling modulates 5-HT release as well as the function and expression of various 5-HT receptors, such as 5-HT_{1A} and 5-HT₂ receptors. At the level of the cell body area, eCBs, via the activation CB1 receptors, control the excitability of DR 5-HT neurons by modulating the strength and plasticity of synaptic transmission. An important finding of these studies is that DR 5-HT neurons can synthesize and release eCBs in an activity-dependent manner. The eCBs released from 5-HT neurons play a central role in mediating retrograde modulation of synaptic transmission in the DR. As such, an alteration in the firing activity of DR 5-HT neurons is likely to induce changes in eCB signaling and synaptic strength in the DR 5-HT neurons. The eCB-mediated DSE in DR 5-HT neurons can be used as a physiological assay to examine how various pharmacological treatments or behavioral manipulations known to alter the excitability of 5-HT neurons can modulate eCB signaling in the DR. For example, it is known that selective serotonin uptake inhibitors (SSRIs) as well as 5-HT_{1A} receptor agonists reduce the excitability of DR 5-HT neurons (Haj-Dahmane et al., 1991; Bèïque et al., 2000). Thus, it will be interesting to examine whether the inhibitory effect induced by these pharmacological agents is accompanied by an alteration of eCB signaling in the DR. More importantly, because 5-HT_{1A} receptor agonists are widely used for the treatment of stress-related mood disorders, elucidating their effects on the eCB signaling will determine the potential contribution of the eCB system in the therapeutic effects of 5-HT_{1A} agonists. Finally, although a number of studies have shown that exposure to various stressors alters the function of DR 5-HT neurons (Baratta et al., 2009; Kirby et al., 2007) and that eCBs regulate stress responses, in part via the modulation of the 5-HT system (Griebel et al., 2005), very little is known about the effects and the mechanisms by which stress affects eCB signaling in DR 5-HT neurons. Future studies are needed to address these issues and enhance the current understanding of the impact of stress on the functional cross talk between eCB and 5-HT systems.

Acknowledgments

This work was supported in part by research grants from the National Institutes of Health research grant MH 078009 to S. HD and AA12435 to R-Y. S. We wish to thank Angela Monaco for reading the manuscript.

References

- Arevalo C, De Miguel R, Hernandez-Tristan R. Cannabinoid effects on anxiety-related behaviors and hypothalamic neurotransmitters. *Pharmacol Biochem Behav.* 2001; 70:123–131. [PubMed: 11566149]
- Aso E, Renoir T, Mengod G, Ledent C, Hamon M, Maldonado R, Lanfumey L, Valverde O. Lack of CB1 receptor activity impairs serotonergic negative feedback. *J Neurochem.* 2009; 109:935–944. [PubMed: 19302195]
- Auclair N, Otani S, Soubrie P, Crepel F. Cannabinoids modulate synaptic strength and plasticity at glutamatergic synapses of rat prefrontal cortex. *J Neurophysiol.* 2000; 83:3287–3293. [PubMed: 10848548]
- Azad SC, Eder M, Marsicano G, Lutz B, Zieglgansberger W, Rammes G. Activation of cannabinoid receptor type 1 decreases glutamatergic and GABAergic synaptic transmission in the lateral amygdala of the mouse. *Learn Mem.* 2003; 10:116–128. [PubMed: 12663750]
- Bacci A, Huguenard JR, Prince DA. Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. *Nature.* 2004; 431:312–316. [PubMed: 15372034]
- Bambico FR, Cassano T, Dominguez-Lopez S, Katz N, Walker CD, Piomelli D, Gobbi G. Genetic deletion of fatty acid amide hydrolase alters emotional behavior and serotonergic transmission in the dorsal raphe, prefrontal cortex, and hippocampus. *Neuropsychopharmacol.* 2010; 35:2083–2100.
- Bambico FR, Katz N, Debonnel G, Gobbi G. Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. *J Neurosci.* 2007; 27:11700–11711. [PubMed: 17959812]
- Baratta MV, Zarza CM, Gomez DM, Campeau S, Watkins LR, Maier SF. Selective activation of dorsal raphe nucleus-projecting neurons in the ventral medial prefrontal cortex by controllable stress. *Eur J Neurosci.* 2009; 30:1111–1116. [PubMed: 19686468]
- Bèïque J, de Montigny C, Blier P, Debonnel G. Effects of sustained administration of the serotonin and norepinephrine reuptake inhibitor venlafaxine: I. in vivo electrophysiological studies in the rat. *Neuropharmacology.* 2000; 39:1800–1812. [PubMed: 10884561]
- Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *Science.* 1997; 277:1094–1097. [PubMed: 9262477]
- Best AR, Regehr WG. Serotonin evokes endocannabinoid release and retrogradely suppresses excitatory synapses. *J Neurosci.* 2008; 28:6508–6515. [PubMed: 18562622]
- Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Matias I, Schiano-Moriello A, Paul P, Williams EJ, Gangadharan U, Hobbs C, Di Marzo V, Doherty P. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J Cell Biol.* 2003; 163:463–468. [PubMed: 14610053]
- Bouaboula M, Poinot-Chazel C, Bourrie B, Canat X, Calandra B, Rinaldi-Carmona M, Le Fur G, Casellas P. Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem J.* 1995; 312:637–641. [PubMed: 8526880]
- Brown RE, Sergeeva OA, Eriksson KS, Haas HL. Convergent excitation of dorsal raphe serotonin neurons by multiple arousal systems (orexin/hypocretin, histamine and noradrenaline). *J Neurosci.* 2002; 22:8850–8859. [PubMed: 12388591]
- Carrasco GA, Van de Kar LD. Neuroendocrine pharmacology of stress. *Eur J Pharmacol.* 2003; 463:235–272. [PubMed: 12600714]
- Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature.* 1997; 389:816–824. [PubMed: 9349813]

- Chávez AE, Chiu CQ, Castillo PE. TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. *Nat Neurosci.* 2010; 13:1511–1518. [PubMed: 21076423]
- Chevalleyre V, Castillo PE. Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron.* 2003; 38:461–472. [PubMed: 12741992]
- Chinetti G, Fruchart JC, Staels B. Peroxisome proliferators-activated receptors (PPARs): Nuclear receptor at the crossroads between lipid metabolism and inflammation. *Inflamm Res.* 2000; 49:497–505. [PubMed: 11089900]
- Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature.* 1996; 384:83–87. [PubMed: 8900284]
- Darmani NA, Janoyan JJ, Kumar N, Crim JL. Behaviorally active doses of the CB1 receptor antagonist SR 141716A increase brain serotonin and dopamine levels and turnover. *Pharmacol Biochem Behav.* 2003; 75:777–787. [PubMed: 12957219]
- Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of cannabinoid receptor in rat brain. *Mol Pharmacol.* 1988; 34:605–613. [PubMed: 2848184]
- Devane WA, Hanus L, Breuer A, Oertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science.* 1992; 258:1946–1949. [PubMed: 1470919]
- Di S, Malcher-Lopes R, Halmos KC, Tasker JG. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. *J Neurosci.* 2003; 23:4850–4857. [PubMed: 12832507]
- Di Marzo V. Endocannabinoids: synthesis and degradation. *Rev Physiol Biochem Pharmacol.* 2008; 160:1–24. [PubMed: 18481028]
- Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, Piomelli D. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature.* 1994; 372:686–691. [PubMed: 7990962]
- Egashira N, Mishima K, Katsurabayashi S, Yoshitake T, Matsumoto Y, Ishida J, Yamaguchi M, Iwasaki K, Fujiwara M. Involvement of 5-hydroxytryptamine neuronal system in Delta (9)-tetrahydrocannabinol-induced impairment of spatial memory. *Eur J Pharmacol.* 2002; 445:221–229. [PubMed: 12079687]
- Egertová M, Cravatt BF, Elphick MR. Comparative analysis of fatty acid amid hydrolase and CB1 cannabinoid receptor expression in the mouse brain: evidence of a widespread role of fatty acid amide hydrolase. *Neuroscience.* 2003; 119:481–496. [PubMed: 12770562]
- Egertová M, Elphick MR. Localization of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB₁. *J Comp Neurol.* 2000; 422:159–171. [PubMed: 10842224]
- Galiègue S, Mary S, Marchand J, Dussosoy D, Carrière D, Carayon P, Bouaboula M, Shire D, Le Fur G, Casellas P. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem.* 1995; 232:54–61. [PubMed: 7556170]
- Gerdeman P, Ronesi J, Lovinger DM. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat Neurosci.* 2002; 5:446–451. [PubMed: 11976704]
- Gingrich JA, Hen R. Dissecting the role of the serotonin system in neuropsychiatric disorders using knockout mice. *Psychopharmacol.* 2001; 155:1–10.
- Gobbi G, Bambico ER, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano M, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proc Natl Acad Sci USA.* 2005; 102:18620–18625. [PubMed: 16352709]
- Goparaju SK, Ueda N, Taniguchi K, Yamamoto S. Enzymes of porcine brain hydrolyzing 2-arachidonoylglycerol an endogenous ligand of cannabinoid receptors. *Biochem Pharmacol.* 1999; 57:417–423. [PubMed: 9933030]

- Griebel G, Stemmelin J, Scatton B. Effects of the cannabinoid CB1 receptor antagonist rimonabant in models of emotional reactivity in rodents. *Biol Psychiatry*. 2005; 57:261–267. [PubMed: 15691527]
- Grueter BA, Brasnjo G, Malenka RC. Postsynaptic TRPV1 triggers cell type-specific long-term depression in the nucleus accumbens. *Nat Neurosci*. 2010; 13:1519–1525. [PubMed: 21076424]
- Haj-Dahmane S, Hamon M, Lanfumey L. K⁺ channel and 5-hydroxytryptamine1A autoreceptor interaction in the rat dorsal raphe nucleus: an *in vitro* electrophysiological study. *Neuroscience*. 1991; 41:495–505. [PubMed: 1714550]
- Haj-Dahmane S, Shen RY. The wake-promoting peptide orexin-B inhibits glutamatergic transmission to dorsal raphe nucleus serotonin neurons through retrograde endocannabinoid signaling. *J Neurosci*. 2005; 25:896–905. [PubMed: 15673670]
- Haj-Dahmane S, Shen RY. Endocannabinoids suppress excitatory synaptic transmission to dorsal raphe serotonin neurons through the activation of presynaptic CB₁ receptors. *J Pharmacol Exp Ther*. 2009; 331:186–196. [PubMed: 19592666]
- Haj-Dahmane S, Shen RY. Regulation of plasticity of glutamate synapses by endocannabinoids and the cyclic-AMP/protein kinase A pathway in midbrain dopamine neurons. *J Physiol*. 2010; 588:2589–2604. [PubMed: 20498231]
- Hall W, Solowij N. Adverse effects of cannabis. *Lancet*. 1998; 352:1611–1616. [PubMed: 9843121]
- Haller J, Varga B, Ledent C, Barna I, Freund T. Context-dependent effects of CB1 cannabinoid gene disruption on anxiety-like and social behavior in mice. *Eur J Neurosci*. 2004a; 16:1906–1912.
- Haller J, Varga B, Ledent C, Freund T. CB1 cannabinoid receptors mediate anxiolytic effects: convergent genetic and pharmacological evidence with CB1-specific agents. *Behav Pharmacol*. 2004b; 15:299–304. [PubMed: 15252281]
- Heifets BD, Castillo PE. Endocannabinoid signaling and long-term synaptic plasticity. *Annu Rev Physiol*. 2009; 71:283–306. [PubMed: 19575681]
- Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autographic study. *J Neurosci*. 1991; 11:563–583. [PubMed: 1992016]
- Hill, MN.; McLaughlin, RJ.; Viau, V.; Gorzalka, BB.; Hillard, CJ. Stress-induced alterations in limbic endocannabinoid content in the rat: correlations to HPA axis activation. 17th Annual Symposium of the International Cannabinoid Research Society; 2007. p. 2007
- Hillard CJ, Edgemond WS, Jarrahian A, Campbell WB. Accumulation of N-arachidonylethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. *J Neurochem*. 1997; 69:631–638. [PubMed: 9231721]
- Kano M, Ohno-Shosaku T, Hashimoto Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev*. 2009; 89:309–380. [PubMed: 19126760]
- Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D. Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med*. 2003; 9:76–81. [PubMed: 12461523]
- Kirby LG, Pan YZ, Freeman-Daniels E, Rani S, Nunan JD, Akanwa A, Beck SG. Cellular effects of swim stress in the dorsal raphe nucleus. *Psychoneuroendocrinology*. 2007; 32:712–723. [PubMed: 17602840]
- Kozak KR, Rowlinson SW, Marnett LJ. Oxygenation of the endocannabinoid, 2-arachidonylglycerol, to glyceryl prostaglandins by cyclooxygenase-2. *J Biol Chem*. 2000; 275:33744–33749. [PubMed: 10931854]
- Kreitzer AC, Regehr WG. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto the purkinje cells. *Neuron*. 2001; 29:717–727. [PubMed: 11301030]
- Lanfumey L, Mongeau R, Cohen-Salmon C, Hamon M. Corticosteroid-serotonin interactions in the neurobiological mechanisms of stress-related disorders. *Neurosci Biobehav Rev*. 2008; 32:1174–1184. [PubMed: 18534678]
- Lehrke M, Lazar MA. The many faces of PPARgamma. *Cell*. 2005; 123:993–999. [PubMed: 16360030]

- Liu RJ, van den Pol AN, Aghajanian GK. Hyocretins (orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory direct actions. *J Neurosci.* 2002; 22:9453–9464. [PubMed: 12417670]
- Mackie K, Hille B. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci USA.* 1992; 89:3825–3829. [PubMed: 1315042]
- Mackie K, Lai Y, Westenbroek R, Mitchell R. Cannabinoids activate an inward rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *J Neurosci.* 1995; 15:6552–6561. [PubMed: 7472417]
- Maejima T, Hashimoto K, Yoshida T, Aiba A, Kano M. Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoids receptors. *Neuron.* 2001; 31:463–475. [PubMed: 11516402]
- Malone DT, Taylor DA. Involvement of somatodendritic 5-HT (1A) receptors in Delta (9)-tetrahydrocannabinol-induced hypothermia in the rat. *Pharmacol Biochem Behav.* 2001; 69:595–601. [PubMed: 11509221]
- Marco EM, Perez-Alvarez L, Borcel E, Rubio M, Guaza C, Ambrosio E, File SE, Viveros MP. Involvement of 5-HT1A receptors in behavioral effects of Cannabinoid receptor agonist CP 55,940 in male rats. *Behav Pharmacol.* 2004; 15:21–27. [PubMed: 15075623]
- Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, Bodor AL, Muccioli GG, Hu SSJ, Woodruff G, Fung S, Lafourcade M, Alexander JP, Long JZ, Xu C, Möller T, Mackie K, Manzoni OJ, Cravatt BF, Stella N. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat Neurosci.* 2010; 13:651–957. [PubMed: 20498681]
- Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgänsberger W, Di Marzo V, Lutz B. The endogenous cannabinoid system controls extinction of aversive memories. *Nature.* 2002; 418:530–534. [PubMed: 12152079]
- Mato S, Aso E, Martin M, Valverde O, Maldonado R, Pazos A. Cb1 knockout mice display impaired functionality of 5-HT1A and 5-HT2A /C receptors. *J Neurochem.* 2007; 103:2111–2120. [PubMed: 17944876]
- Matsuda LA, Bonner TL, Lolait SJ. Localization of cannabinoid receptor mRNA in rat brain. *J Comp Neurol.* 1993; 327:535–550. [PubMed: 8440779]
- Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature.* 1990; 346:561–564. [PubMed: 2165569]
- Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z. Identification of endogenous 2-monoglyceride, present in canine gut that binds to cannabinoid receptors. *Biochem Pharmacol.* 1995; 50:83–90. [PubMed: 7605349]
- Mendiguren A, Pineda J. Effect of the CB1 receptor antagonists rimonabant and AM251 on the firing rate of dorsal raphe nucleus neurons in rat brain slices. *Br J Pharmacol.* 2009; 158:1579–1587. [PubMed: 19845674]
- Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, Grimaldi PA, Kadowaki T, Lazar MA, O’Rahilly S, Palmer CN, Plutzky J, Reddy JK, Spielgelman BM, Staels B, Wahli W. International Union of Pharmacology. LXI Peroxisome proliferators-activated receptors. *Pharmacol Rev.* 2006; 58:726–741. [PubMed: 17132851]
- Moranta D, Esteban S, Garcia-Sevilla JA. Chronic treatment and withdrawal of the cannabinoid agonist WIN 55,212-2 modulate the sensitivity of presynaptic receptors involved in the regulation of monoamine syntheses in rat brain. *Naunyn-Schmiedeberg’s Arch Pharmacol.* 2009; 379:61–72.
- Morgan NH, Stanford IM, Woodhall GL. Functional CB2 type cannabinoid receptors at CNS synapses. *Neuropharmacology.* 2009; 57:356–368. [PubMed: 19616018]
- Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993; 365:61–65. [PubMed: 7689702]
- Murillo-Rodriguez E, Millán-Aldaco D, Di Marzo V, Drucker-Colin R. The anandamide membrane transporter inhibitor, VDM-11 modulates sleep and c-Fos expression in the rat brain. *Neuroscience.* 2008; 157:1–11. [PubMed: 18822353]
- Nakazi M, Bauer U, Nickel T, Kathmann M, Schlicker E. Inhibition of serotonin release in the mouse brain via presynaptic CB receptors. *Naunyn-Schmiedeberg’s Arch Pharmacol.* 2000; 361:19–24.

- Nemeroff CB. Anxiolytics: past, present, and future agents. *J Clin Psychiatry*. 2003; 64 (3):3–6. [PubMed: 12662127]
- Ohno-Shosaku T, Maejima T, Kano M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron*. 2001; 29:729–739. [PubMed: 11301031]
- Patel S, Roelke CT, Radmecher DJ, Cullina WE, Hillard CJ. Endocannabinoid signaling negatively regulate stress-induced activation of the hypothalamic-pituitary-adrenal axis. *Endocrinology*. 2004; 145:5431–5438. [PubMed: 15331569]
- Qi L, Jacob A, Wang P, Wu R. Peroxisome proliferator activated receptor- γ and traumatic brain injury. *Int J Clin Exp Med*. 2010; 3:283–292. [PubMed: 21072262]
- Robbe D, Koft M, Remaury A, Bockaert J, Manzoni OJ. Endogenous cannabinoids mediate long-term-depression in the nucleus accumbens. *Proc Natl Acad Sci USA*. 2002; 99:8384–8388. [PubMed: 12060781]
- Resstel LBM, Tavares RF, Lisboa SFS, Joca SRL, Corrêa FMA, Guimarães FS. 5-HT_{1A} receptors are involved in the cannabidiol-induced attenuation of behavioral and cardiovascular responses to acute restraint stress in rats. *Br J Pharmacol*. 2009; 156:181–188. [PubMed: 19133999]
- Shen M, Piser TM, Seybold VS, Thayer SA. Cannabinoid receptor agonists inhibit glutamergic synaptic transmission in rat hippocampal culture. *J Neurosci*. 1996; 16:4322–4334. [PubMed: 8699243]
- Steiner MA, Marsicano G, Nestler EJ, Holsboer F, Lutz B, Wotjak CT. Antidepressant-like behavioral effects of impaired cannabinoid receptor type 1 signaling coincide with exaggerated corticosterone secretion in mice. *Psychoneuroendocrinology*. 2008; 33:54–62. [PubMed: 17976922]
- Stella N, Schweitzer P, Piomelli D. A second endogenous cannabinoid that modulates long-term potentiation. *Nature*. 1997; 388:773–778. [PubMed: 9285589]
- Sugiura T, Kondo S, Sukagawa A, Tonegawa T, Nakane S, Yamashita A, Waku K. Enzymatic synthesis of anandamide, an endogenous cannabinoid receptor ligand, through N-acylphosphatidylethanolamine pathway in testis: involvement of Ca(2+)-dependent transacylase and phosphodiesterase activities. *Biochem Biophys Res Commun*. 1996; 218:113–117. 97. [PubMed: 8573114]
- Sun Y, Alexander SP, Garle MJ, Gibson CL, Hewitt K, Murphy SP, Kendall DA, Bennett AJ. Cannabinoid activation of PPAR α ; a novel neuroprotective mechanism. *Br J Pharmacol*. 2007; 152:734–743. [PubMed: 17906680]
- Szabo B, Dorner L, Pfreundtner C, Norenberg W, Starke K. Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. *Neuroscience*. 1998; 85:395–403. [PubMed: 9622239]
- Tanimura A, Yamazaki A, Hashimoto Y, Uchigashima M, Kawata S, Abe M, Kita Y, Hashimoto K, Shimizu T, Watanabe M, Sakimura K, Kano M. The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase α mediates retrograde suppression of synaptic transmission. *Neuron*. 2010; 65:320–327. [PubMed: 20159446]
- Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipi-activated transcription factor. *Cell*. 1994; 79:1147–1156. [PubMed: 8001151]
- Tóth A, Blumberg PM, Boczán J. Anandamide and the vanilloid receptor (TRPV1). *Vitam Horm*. 2009; 81:389–419. [PubMed: 19647120]
- Tournier M, Sorbara F, Gindre C, Swendsen JD, Verdoux H. Cannabis use and anxiety in daily life: a naturalistic investigation in a non-clinical population. *Psychiatry Res*. 2003; 118:1–8. [PubMed: 12759155]
- Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience*. 1998; 83:393–411. [PubMed: 9460749]
- Tzavara ET, Davis RJ, Perry KW, Li X, Salhoff C, Bymaster FP, Witkin JM, Nomikos GG. The CB1 receptor antagonist SR 141716A selectively increases monoaminergic neurotransmission in the medial prefrontal cortex: implications for therapeutic actions. *Br J Pharmacol*. 2003; 138:544–553. [PubMed: 12598408]

- Uriguen I, Perez-Rial S, Ledent C, Palomo T, Manzanares J. Impaired action of anxiolytic drugs in mice deficient in cannabinoid Cb1 receptors. *Neuropharmacology*. 2004; 46:966–973. [PubMed: 15081793]
- Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Mernett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA. Identification and functional characterization of brainstem cannabinoid CB₂ receptors. *Science*. 2005; 31:329–332. [PubMed: 16224028]
- Venkatachalam K, Montel C. TRP channels. *Annu Rev Biochem*. 2007; 76:387–417. [PubMed: 17579562]
- Viveros MP, Marco EM, File SE. Endocannabinoid system and stress and anxiety responses. *Pharmacol Biochem Behav*. 2005; 81:331–342. [PubMed: 15927244]
- Wada K, Nakajima A, Katayama K, Kudo C, Shibuya A, Kubota N, Terauchi Y, Tachibana M, Miyoshi H, Kamisaki Y, Mayumi T, Kadowaka T, Blumberg RS. Peroxisome proliferators-activated receptor gamma-mediated regulation of neuronal stem proliferation and differentiation. *J Biol Chem*. 2006; 281:12673–12681. [PubMed: 16524877]
- Ward SJ, Lefever TW, Jackson C, Tallarida RJ, Walker EA. Effects of a cannabinoid 1 receptor antagonist and serotonin 2C receptor agonist alone and in combination on motivation for palatable food: a dose-addition analysis study in mice. *J Pharmacol Exp Ther*. 2008; 325:567–576. [PubMed: 18256173]
- Williamson EM, Evans FJ. Cannabinoids in clinical practice. *Drugs*. 2000; 60:1303–1314. [PubMed: 11152013]
- Wilson RI, Kunos G, Nicoll RA. Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron*. 2001; 31:453–462. [PubMed: 11516401]
- Zavitsanou K, Wang H, Dalton VS, Nguyen V. Cannabinoid administration increases 5HT1A receptor binding and mRNA expression in the hippocampus of adult but not adolescent rats. *Neuroscience*. 2010; 169:315–324. [PubMed: 20438810]
- Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sergård M, Di Marzo V, Julius D, Högestätt ED. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature*. 1999; 400:452–457. [PubMed: 10440374]

Figure 1. Model of eCB modulation of 5-HT neurotransmission

CB1 receptors are expressed by local glutamate neurons and by glutamatergic inputs from forebrain areas such as, the prefrontal cortex that target 5-HT neurons and GABA interneurons of the DR. Activation of these receptors either by exogenous cannabinoids or eCB released from DR 5-HT neurons reduces the strength of glutamate GABA synapses impinging on DR 5-HT neurons. The overall effect of eCB signaling on the electrical activity of DR 5-HT will depend on the balance of excitation and inhibition in the DR. CB1 receptors are also located on 5-HT terminals, activation of these receptors reduces 5-HT release and overall 5-HT neurotransmission.