Neuropsychopharmacology REVIEWS (2015), 1–23

Neurobiological Interactions Between Stress and the Endocannabinoid System

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Stress affects a constellation of physiological systems in the body and evokes a rapid shift in many neurobehavioral processes. A growing body of work indicates that the endocannabinoid (eCB) system is an integral regulator of the stress response. In the current review, we discuss the evidence to date that demonstrates stress-induced regulation of eCB signaling and the consequential role changes in eCB signaling have with respect to many of the effects of stress. Across a wide array of stress paradigms, studies have generally shown that stress evokes bidirectional changes in the two eCB molecules, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), with stress exposure reducing AEA levels and increasing 2-AG levels. Additionally, in almost every brain region examined, exposure to chronic stress reliably causes a downregulation or loss of cannabinoid type 1 (CB1) receptors. With respect to the functional role of changes in eCB signaling during stress, studies have demonstrated that the decline in AEA appears to contribute to the manifestation of the stress response, including activation of the hypothalamic–pituitary–adrenal (HPA) axis and increases in anxiety behavior, while the increased 2-AG signaling contributes to termination and adaptation of the HPA axis, as well as potentially contributing to changes in pain perception, memory and synaptic plasticity. More so, translational studies have shown that eCB signaling in humans regulates many of the same domains and appears to be a critical component of stress regulation, and impairments in this system may be involved in the vulnerability to stress-related psychiatric conditions, such as depression and posttraumatic stress disorder. Collectively, these data create a compelling argument that eCB signaling is an important regulatory system in the brain that largely functions to buffer against many of the effects of stress and that dynamic changes in this system contribute to different aspects of the stress response.

Neuropsychopharmacology Reviews advance online publication, 8 July 2015; doi:10.1038/npp.2015.166

INTRODUCTION

Basic Primer on Stress

The stress response is a biological cascade of events that occurs in response to a real or perceived threat to homeostasis. It requires the coordinated activation of a constellation of physiological systems that act to promote the survival of the organism. Typically, the response to physiological and/or psychological stressors requires the concerted activation of two parallel responses: an autonomic response and a neuroendocrine response. The autonomic response involves stimulation of sympathetic motor and hormonal outputs via descending neural circuits originating in hypothalamic preautonomic control centers and results in the release of catecholamines within the brain and circulation. The neuroendocrine stress response is mediated by activation of the hypothalamic–pituitary–adrenal (HPA) axis, which results in an increase in circulating corticosteroids that target multiple organ systems (Pecoraro *et al*, 2006). Stress exposure also provokes a shift in many neurobehavioral processes, such as anxiety/vigilance, memory, reward salience, pain sensitivity, and coping behaviors (McEwen, 2012a). Collectively, these changes in biological function produce coordinated and highly adaptive responses that are conducive to survival in response to a threat.

Decades of basic and clinical research have delineated several well-defined brain circuits that are important for the manifestation of the physiological response to psychological stressors. Although these circuits have been discussed at great length in more focused reviews (see Hermans *et al*,

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Received 12 March 2015; revised 20 May 2015; accepted 20 May 2015; accepted article preview online 12 June 2015

2014b; Sparta *et al*, 2013; Ulrich-Lai and Herman, 2009), we will outline those relevant to the current review. In brief, sensory information regarding the external environment is processed by the thalamus and primary sensory cortical centers and funneled to the amygdala through a network of corticothalamic afferents. Of particular importance to stress is the transmission of information to nuclei of the amygdala and extended amygdala where preconscious threat detection occurs, emotional valence is ascribed, and reference to previous experiences occurs through crosstalk with the medial prefrontal cortex (mPFC) and hippocampus (Bishop, 2008; Hermans *et al*, 2014a; Likhtik and Paz, 2015; Roozendaal *et al*, 2009). This triadic circuit of the amygdala–mPFC–hippocampus has been found to be relevant for almost every neurobehavioral response to psychological stress (McEwen, 2012a). In general, activation of output pyramidal neurons of the basolateral amygdala (BLA) contributes to many aspects of stress, including HPA axis activation, anxiety, pain sensitivity, and alterations in cognitive processes through the trans-synaptic recruitment of downstream circuits, such as the central amygdala (CeA), medial amygdala, bed nucleus of the stria terminalis (BNST), nucleus accumbens, and distinct hypothalamic nuclei such as the lateral, anterior, and dorsomedial hypothalamus (Hermans *et al*, 2014b; Janak and Tye, 2015; Ulrich-Lai and Herman, 2009; Veinante *et al*, 2013). Conversely, excitatory inputs from the mPFC and hippocampus dampen amygdala output (Hubner *et al*, 2014; Likhtik *et al*, 2005). Consistent with this, damage to either of these structures typically results in amplified responses to stress and impairments in termination of the stress response (McEwen, 2012b; Radley, 2012), and in humans, reduced functional connectivity of these circuits, or hyperactivity of the amygdala, results in increased anxiety and sensitivity to stress (Kim *et al*, 2011; Pruessner *et al*, 2010; Swartz *et al*, 2015). Given the demonstrated importance of these circuits in the regulation of the neurobehavioral effects of stress, this review will focus on the mechanisms and functional contributions endocannabinoid (eCB) signaling has within these circuits with respect to the regulation of various aspects of the stress response.

Basic Primer on the eCB System

The eCB system nomenclature derives from the finding that eCBs and plant-derived cannabinoids converge on a common molecular receptor target (Mechoulam and Parker, 2013). The eCB system is a neuromodulatory lipid system, which consists of the cannabinoid receptor type 1 and type 2 (CB1 and CB2 receptors, respectively; Devane *et al*, 1992; Herkenham *et al*, 1990; Matsuda *et al*, 1990) and two major endogenous ligands, *N*-arachidonyl ethanolamine (anandamide, AEA; Devane *et al*, 1992) and 2-arachidonoyl glycerol (2-AG; Sugiura *et al*, 1995). CB receptors are the primary molecular target of plant-derived tetrahydrocannabinol and couple to $G_{i/o}$ proteins that function to inhibit adenylyl cyclase activity, activate potassium channels, and inhibit voltage-gated calcium channels (Howlett *et al*, 2002). As CB1 receptors are primarily localized to axon terminals, activation of these receptors results in a robust suppression of neurotransmitter release into the synapse (Kano *et al*, 2009; Figure 1). CB1 receptors not only represent the most abundant class of G-protein-coupled receptors in the central nervous system (Herkenham *et al*, 1990) but are also present in a variety of peripheral tissues (Howlett *et al*, 2002). Within the brain, CB1 receptors are expressed on GABAergic, glutamatergic, serotonergic, noradrenergic, and dopaminergic terminals (Azad *et al*, 2008; Haring *et al*, 2007; Hermann *et al*, 2002; Kano *et al*, 2009; Morozov *et al*, 2009; Oropeza *et al*, 2007), but given the relative abundance of excitatory and inhibitory neurons in the brain, and the high levels of CB1 receptor expression on these terminals, the predominant effects of eCB signaling occur at GABAergic and glutamatergic synapses (Katona and Freund, 2012). CB2 receptors are mostly located in immune cells and, when activated, can modulate immune cell migration and cytokine release both outside and within the brain (Pertwee, 2005). There is also evidence that they are possibly expressed by some neurons (Atwood *et al*, 2012; Van Sickle *et al*, 2005), but the role of these putative neuronal CB2 receptors has yet to be established (Atwood and Mackie, 2010). In addition, some eCB ligands are active at other receptor targets, including peroxisome proliferator-activated receptor and transient receptor potential vanilloid type 1, and can also directly affect activity of some ion channels (Di Marzo and Petrocellis, 2012).

AEA and 2-AG are synthesized 'on demand' from phospholipid precursors in the postsynaptic membrane by Ca^{2+}-dependent and -independent mechanisms (Kano *et al*, 2009) and feedback in a retrograde manner onto presynaptic terminals, thus suppressing afferent neurotransmitter release via activation of CB1 receptors (Ohno-Shosaku and Kano, 2014). The process by which this release and transmission across the synapse occurs still remains enigmatic, but the collective electrophysiological and biochemical evidence to date strongly supports a model of postsynaptic synthesis and a presynaptic site of action (see Figure 1). The synthesis of 2-AG is tightly coupled to the generation of diacylglycerol from phospholipase C activity, which is rapidly converted to 2-AG by the enzyme diacylglycerol lipase (Bisogno *et al*, 2003; Sugiura *et al*, 1995; Figure 1). The synthesis of AEA, on the other hand, is far less clear and appears to be performed by at least three redundant pathways, none of which have been verified as the primary source of AEA within the brain (Ahn *et al*, 2008; Figure 1). Following release into the synaptic cleft, AEA and 2-AG are subsequently taken back into the cell by a still poorly defined uptake process mediated by a transporter mechanism (Fu *et al*, 2011; Hillard *et al*, 1997) and primarily degraded by distinct hydrolytic enzymes, the fatty acid amide hydrolase (FAAH; Cravatt *et al*, 2001) and monoacylglycerol lipase (MAGL; Dinh *et al*, 2002), respectively (Figure 1). These two degrading enzymes display distinct subcellular localization, suggesting different signaling properties for AEA and 2-AG (Cristino *et al*, 2008;

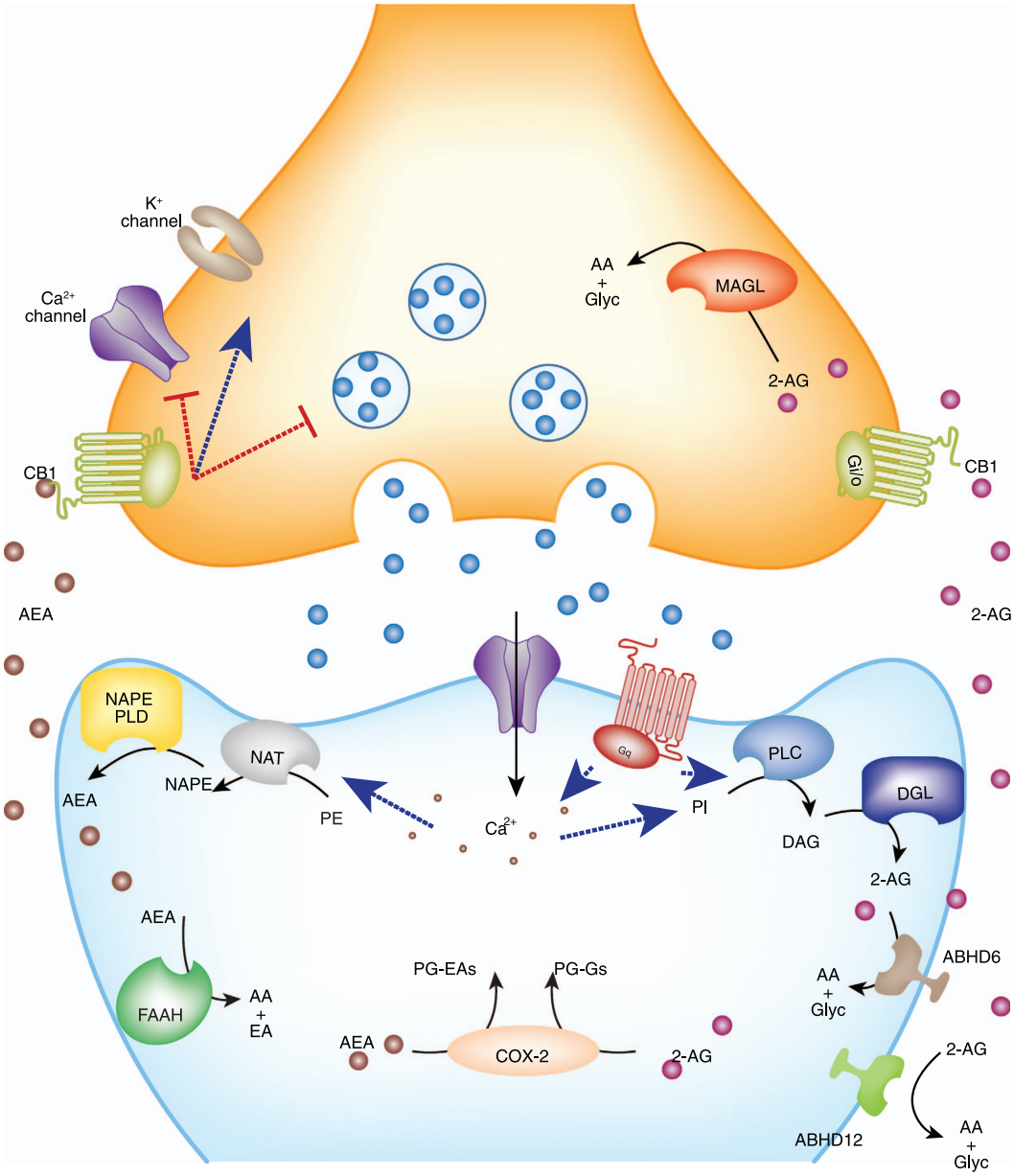

Figure 1. General model illustrating the retrograde endocannabinoid signaling. Upon release of neurotransmitter (eg, glutamate), postsynaptic depolarization causes increased intracellular Ca^{2+} levels through activation of AMPA, NMDA receptors and/or Gq-coupled receptors (eg, mGluR1/5) and voltage-gated Ca^{2+} channels. Intracellular Ca^{2+} elevation increases endocannabinoid biosynthesis, although there is evidence for Ca^{2+}-independent forms of endocannabinoid synthesis as well. This model illustrates the two primary biosynthetic pathways for anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), respectively. AEA is synthesized from phospholipid precursors (eg phosphatidylethanolamine, PE) by a Ca^{2+}-dependent transacylase, N-acyltransferase (NAT), yielding N-arachidonoyl PE (NAPE). NAPE is then hydrolyzed by a phospholipase D (NAPE-PLD) to yield AEA. Ca^{2+} influx and/or the activation of G_q-coupled receptors stimulate phospholipase C (PLC), which hydrolyses phosphatidylinositol (PI) into diacylglycerol (DAG). DAG is converted to 2-AG by diacylglycerol lipase (DGL). AEA and 2-AG then migrate from postsynaptic neurons to bind presynaptic-located cannabinoid type 1 (CB1) receptors. Once activated, CB1 receptors couple through $G_{i/o}$ proteins to inhibit adenylyl cyclase and regulate ion channels and ultimately suppress neurotransmitter release. Endocannabinoid signaling is then terminated by degrading enzymes. AEA is mainly hydrolyzed to arachidonic acid (AA) and ethanolamine (EA) by fatty acid amide hydrolase (FAAH), located postsynaptically. 2-AG is hydrolyzed presynaptically to AA and glycerol (Glyc) by monoacylglycerol lipase (MAGL), which accounts for ~85% of 2-AG hydrolysis, and postsynaptically by alpha-beta-hydrolase 6/12 (ABHD6/12), which accounts for the remainder of 2-AG hydrolysis. AEA and 2-AG are also oxygenated by cyclo-oxygenase 2 (COX-2) to form prostaglandin-ethanolamides (PG-EAs) and prostaglandin-glycerols (PG-Gs).

Kano *et al*, 2009). All studies to date have indicated that FAAH is predominantly located on intracellular membranes in postsynaptic cells, while MAGL is positioned in close proximity of CB1 receptors, in presynaptic terminals, at least within the brain regions that have been examined to date such as the hippocampus, amygdala, and cerebellum (Gulyas *et al*, 2004). In addition to these two primary metabolic enzymes, both AEA and 2-AG are also oxygenated by cyclo-

oxygenase 2 (COX-2) to form bioactive prostaglandin derivatives (Hermanson *et al*, 2013; Figure 1). Additionally, a small proportion of 2-AG is also metabolized by the alpha-beta hydrolase (ABHD) class of enzymes, specifically ABHD6 and ABHD12 (Blankman *et al*, 2007; Marrs *et al*, 2010; Figure 1). The functional role of these alternate metabolic pathways is not well characterized. For instance, due to its postsynaptic localization (Blankman *et al*, 2007; Marrs *et al*, 2010), it is likely that ABHD6 might be involved in the regulation of 2-AG levels released into the synaptic cleft. However, to date, studies have clearly identified the physiological significance of FAAH and MAGL as regulators of eCB levels as pharmacological or genetic inactivation of these two enzymes results in profound accumulation of AEA and 2-AG, respectively (Cravatt *et al*, 2001; Long *et al*, 2009).

In general, the activation of the eCB system at the synapse leads to a short or a sustained suppression of neurotransmitter release from the presynaptic compartment. Despite the fact that both AEA and 2-AG similarly act to regulate presynaptic transmitter release, it is believed that these two molecules of the eCB system may operate in phasic and tonic modes, thereby differentially mediating homeostatic, short-term, and long-term synaptic plasticity processes throughout the brain (Ahn *et al*, 2008; Hill and Tasker, 2012; Katona and Freund, 2012). It is thought that AEA represents the 'tonic' signaling molecule of the eCB system that acts to regulate basal synaptic transmission, whereas 2-AG represents the 'phasic' signal activated during sustained neuronal depolarization and mediates many forms of synaptic plasticity (Ahn *et al*, 2008; Gorzalka *et al*, 2008; Katona and Freund, 2012).

There are several lines of evidence suggesting that the eCB system may be an important regulator of various aspects of the stress response. First, there is significant evidence within human populations that cannabis consumption typically results in a reduction of perceived stress, an increase in relaxation, and a dampening of feelings of anxiety (Green *et al*, 2003). Given that the physiological actions of cannabis are mediated by activation of the CB1 receptor, this suggested that a normative function of the eCB system could be to dampen or buffer against the effects of stress. Consistent with this hypothesis, pharmacological or genetic disruption of eCB signaling reliably produces a neurobehavioral phenotype, which directly parallels the classical manifestation of a stress response, including activation of the HPA axis, increased anxiety, suppressed feeding behavior, reduced responsiveness to rewarding stimuli, hypervigilance and arousal, enhanced grooming behavior, and impaired cognitive flexibility (Bellocchio *et al*, 2013; Friemel *et al*, 2014; Haller *et al*, 2004; Marsicano *et al*, 2002; Patel *et al*, 2004; Sanchis-Segura *et al*, 2004; Santucci *et al*, 1996; Shonesy *et al*, 2014; Tallett *et al*, 2007; Varvel and Lichtman, 2002). As such, these data indicate that there is a prominent stress-inhibitory role of the eCB system. Anatomically, within the cortico-limbic circuit that regulates the stress response, eCB synthetic and degradative enzymes and CB1 receptors are prominently expressed in the amygdala (primarily in the BLA but also in the central nucleus as well; Ramikie *et al*, 2014; Ramikie and Patel, 2012), hippocampus, mPFC, and nucleus accumbens (Herkenham *et al*, 1991; Marsicano and Kuner, 2008; McPartland *et al*, 2007), where they modulate both excitatory and inhibitory signaling within specific neuronal circuits. For the purposes of the current review, we will focus on the role of the CB1 receptor and the eCB molecules AEA and 2-AG within discrete regions of this cortico-limbic circuit and how they interact with stress within the brain to modulate various aspects of the stress response.

Stress Exposure Modulates the eCB System

One of the initial lines of evidence suggesting that the eCB system may be involved in the stress response was the fact that this system was very reliably modulated by exposure to stress. The effects of stress on the eCB system appear to be quite complex, regionally specific, and time-dependent relative to exposure to stress and the chronicity of stress exposure. For the sake of clarity, we will discuss the nature in which stress modulates each component of the eCB system.

Effects of Acute Stress on AEA Brain Levels

Within the examined subcortical–limbic structures of the brain, exposure to acute stress generally causes a rapid reduction in tissue content of AEA in response to an array of psychological stressors. Within the amygdala, this effect appears to be quite prominent as exposure of both rats and mice to restraint stress causes a reduction in the tissue levels of AEA (Gray *et al*, 2015; Hill *et al*, 2009c; Patel *et al*, 2005b; Rademacher *et al*, 2008). Similarly, both acute restraint stress and social defeat stress have been found to reduce AEA content within the hippocampus (Dubreucq *et al*, 2012; Wang *et al*, 2012b). This reduction in AEA content is, at least in part, mediated by an increase in AEA hydrolysis by FAAH as acute stress increases FAAH activity within the amygdala (Gray *et al*, 2015; Hill *et al*, 2009c). Similarly, in the hippocampus, acute restraint stress increases FAAH protein levels 24 h after the stress exposure (Navarria *et al*, 2014). Interestingly, this effect appears to be conserved throughout the lifespan as early life stress in the form of maternal separation has also been shown to reduce AEA content within the hippocampus (Marco *et al*, 2013). Unlike the consistency seen in the amygdala and hippocampus, the mPFC seems to be somewhat of a more complex structure as exposure to swim stress has been found to produce a robust reduction of AEA content (McLaughlin *et al*, 2012), but neither acute restraint (Gray *et al*, 2015; Hill *et al*, 2011b; Rademacher *et al*, 2008) nor acute social defeat (Dubreucq *et al*, 2012) were found to have any effect on AEA content in the mPFC. Similarly, restraint stress was found to have no effect on FAAH mRNA, protein, or activity within the mPFC (Gray *et al*, 2015; Navarria *et al*, 2014).

It should be noted that footshock appears to be the one anomalous stressor with respect to the effects on AEA content. Two separate studies have both found that footshock actually elevates, not reduces, AEA levels in the mPFC, amygdala, hippocampus, and periaqueductal gray (Hohmann *et al*, 2005; Morena *et al*, 2014b). It is possible that the different nature of the stressful stimulus, the different behavioral paradigm used, or the additional painful component of footshock activate specific neuronal pathways that mobilize AEA to modulate diverse aspects of the stress response, such as memory (Morena *et al*, 2014b) and pain (Hohmann *et al*, 2005). Interestingly, Bluett *et al* (2014) have recently demonstrated a robust, brain-wide reduction in AEA content 24 h following exposure to footshock. This suggests that, although noxious stimuli, such as footshock, may rapidly elevate AEA content, there still appears to be a delayed reduction in AEA levels resulting in the typical stress-induced 'AEA-deficient' state (Bluett *et al*, 2014).

Mechanistically, the effects of stress on AEA appear to be rapid and may precede the onset of the HPA axis (Gunduz-Cinar *et al*, 2013a; Hill *et al*, 2009c), suggesting that the signal mediating these effects was upstream of glucocorticoid release. Consistent with this, we have recently demonstrated that the neuropeptide corticotropin-releasing hormone (CRH), which is rapidly released in response to stress (Merlo Pich *et al*, 1995; Roozendaal *et al*, 2002), increases FAAH activity following activation of CRH type 1 receptors (CRHR1) on glutamatergic neurons in the BLA (Gray *et al*, 2015). This reduces AEA signaling through a yet to be determined intracellular signaling mechanism. Interestingly, this interaction between CRH and FAAH did not occur within the mPFC (Gray *et al*, 2015), which may explain the unreliable effects of stress on AEA within the mPFC. It should be noted, however, that CRHR1 activation exerts similar effects on intracellular signaling pathways within the amygdala and hippocampus, by increasing pERK1/2 levels in both brain structures, without altering pERK1/2 expression in the CeA, paraventricular nucleus of the hypothalamus (PVN), or neocortex (Refojo *et al*, 2005). Thus this evidence creates a potential platform from which to explore the mechanisms linking stress, CRH, and AEA signaling, and the regional specificity of this interaction. In terms of the recovery of this effect, it has been shown that administration of corticosterone can actually increase AEA levels within the amygdala (Hill *et al*, 2010a), suggesting that this may be part of the feedback loop through which AEA levels normalize following cessation of stress.

Effects of Chronic Stress on AEA Brain Levels

With respect to repeated or chronic stress, the nature of the stress exposure itself seems to have significant impact on changes in AEA content. Similar to what has been reported with exposure to acute stress, repeated exposure to the same stressor (chronic homotypic stress), such as restraint stress and social defeat stress, seems to reliably reduce AEA content in the amygdala (Hill *et al*, 2010b, 2013b; Patel *et al*, 2005b; Rademacher *et al*, 2008), hippocampus (Dubreucq *et al*, 2012; Hill *et al*, 2010b), hypothalamus (Dubreucq *et al*, 2012; Hill *et al*, 2010b), and mPFC (Hill *et al*, 2010b; Rademacher *et al*, 2008). Similar to acute stress, this reduction in AEA has been coupled to an increase in FAAH activity, at least within the amygdala, and FAAH KO mice do not exhibit any changes in AEA content within the amygdala in response to repeated stress (Hill *et al*, 2013b). These effects of chronic homotypic stress on FAAH and AEA signaling are likely mediated by sustained exposure to corticosterone as models of chronic corticosterone exposure have similarly found increases in FAAH activity and reductions in AEA content within the amygdala, hippocampus, and mPFC (Bowles *et al*, 2012, Gray *et al*, unpublished data). However, these effects of corticosterone appear to still be mediated by a CRH mechanism as the ability of chronic corticosterone to increase FAAH and reduce AEA levels are reversed by CRHR1 antagonist and mimicked by forebrain overexpression of CRH (Gray *et al*, unpublished data). As chronic exposure to corticosterone is known to upregulate extra-hypothalamic CRH, at least within the amygdala and extended amygdala (Makino *et al*, 1994a,b; Swanson and Simmons, 1989) and mPFC (Gray *et al*, unpublished data), this would suggest that a common CRH mechanism mediates both the acute and chronic stress effects on FAAH and AEA.

In contrast to homotypic stress, the effects of chronic heterotypic stress on AEA content are less consistent across studies. Although one study found reductions in AEA levels in all brain regions examined (PFC, hippocampus, amygdala, ventral striatum, hypothalamus, and midbrain) following 3 weeks of chronic unpredictable stress (CUS; (Hill *et al*, 2008b) and another found increased FAAH protein expression within the hippocampus following CUS (Reich *et al*, 2009), several other studies have found no effect of CUS on AEA levels in several brain regions, including the striatum, hippocampus, cortex, midbrain, and thalamus (Bortolato *et al*, 2007; Hill *et al*, 2005b; Lomazzo *et al*, 2015; Wang *et al*, 2010) or changes in FAAH activity in any of these brain regions (Hill *et al*, 2008b). It is possible that the discrepancy of these effects could be related to the time point following the cessation of stress when AEA levels were measured; however, an alternate hypothesis is that there are differences between chronic homotypic and heterotypic stressors on CRH signaling. Chronic homotypic stress has been shown to elevate CRH mRNA in the amygdala (Gray *et al*, 2010), similar to what was seen with sustained exposure to corticosterone (Makino *et al*, 1994a,b; Swanson and Simmons, 1989), while CUS surprisingly has been found to have little effects on CRH in the amygdala (Kim *et al*, 2006; Sterrenburg *et al*, 2011) and actually downregulates CRHR1 in the amygdala (Sandi *et al*, 2008). As such, the divergence of the effects of homotypic *vs* heterotypic stress on AEA content could be due to the differential effects of these stress paradigms on CRH signaling; however, given that this relationship has only been established in the amygdala (Gray *et al*, 2015), this model cannot explain the differential effects

in other brain regions. Regardless of the complexity of these findings, the general picture emerging from these studies is that AEA signaling is generally compromised, particularly within the amygdala and hippocampus, following exposure to stress.

Effects of Acute Stress on 2-AG Brain Levels

Unlike the effects of stress on AEA, the majority of studies suggest that stress acts to increase 2-AG signaling. Specifically, acute restraint stress produces moderate increases in 2-AG content within the mPFC (Hill *et al*, 2011b), hippocampus (Wang *et al*, 2012b) and hypothalamus (Evanson *et al*, 2010) while footshock increases 2-AG content in the periaqueductal gray (Hohmann *et al*, 2005). In contrast to these effects, several studies have shown that acute stress does not increase 2-AG content within the amygdala (Hill *et al*, 2009c; Patel *et al*, 2005b, 2009; Rademacher *et al*, 2008), at least at the time point analyzed immediately at the end of a 30-min restraint stress session. Unlike the rapid changes in AEA following stress, the increases in 2-AG show a prominent delay. Specifically, no changes in 2-AG content are evident in the mPFC after a 15-min swim stress session or a 30-min bout of social defeat (Dubreucq *et al*, 2012; McLaughlin *et al*, 2012); increases in 2-AG, however, were detected in PFC 60 min following restraint stress (Hill *et al*, 2011b). These data indicate significant differences in the temporal dynamics of AEA and 2-AG changes following stress exposure. This time lag is consistent with several observations demonstrating that elevated corticosterone levels mediate the increase in 2-AG following stress. For example, the increase in 2-AG content both within the mPFC and hippocampus following stress is blocked by antagonism of the glucocorticoid receptor (Hill *et al*, 2011b; Wang *et al*, 2012b). Similarly, administration of corticosterone alone has been found to increase 2-AG content within the hippocampus (Atsak *et al*, 2012a) and hypothalamus (Di *et al*, 2005; Hill *et al*, 2010a), and corticosterone levels following stress correlate with 2-AG levels in the mPFC (Roberts *et al*, 2012). Given that it takes a minimum of 20 min, and up to 60 min, for corticosterone levels to elevate in the brain (Bouchez *et al*, 2012; Dominguez *et al*, 2014; Heinzmann *et al*, 2010), this temporal lag from stress onset corresponds with the delayed timeline for 2-AG content to increase. Collectively, these data indicate that corticosterone is the primary mediator of increased 2-AG release in response to stress; however, the mechanisms by which corticosterone enhances 2-AG levels are unresolved.

Effects of Chronic Stress on 2-AG Brain Levels

Similar to the mirroring effects of acute and repeated stress on AEA, the effects of chronic stress on 2-AG content are consistent, although more robust, than what is seen following acute stress. Also, similarly to AEA, the magnitude of these effects is very sensitive to whether the chronic stress is homotypic or heterotypic in nature. Chronic homotypic stress, including restraint and social defeat, reliably elevates 2-AG content in the amygdala (Hill *et al*, 2010b; Patel *et al*, 2005b, 2009; Rademacher *et al*, 2008), mPFC (Dubreucq *et al*, 2012; Patel *et al*, 2005b; Rademacher *et al*, 2008), hypothalamus (Dubreucq *et al*, 2012; Patel *et al*, 2004), and hippocampus (Dubreucq *et al*, 2012). Consistent with what was seen with AEA, the increased 2-AG content observed following chronic homotypic stress is largely recapitulated by exposure to chronic corticosterone in the hippocampus (Bowles *et al*, 2012), amygdala (Hill *et al*, 2005a; Gray *et al*, unpublished data), and mPFC (Gray *et al*, unpublished data). One report has demonstrated that chronic stress results in a reduction of MAGL expression at the membrane within the amygdala (where it would most efficiently metabolize 2-AG), suggesting that reduced hydrolysis may account for the increased 2-AG contents seen following chronic homotypic stress (Sumislawski *et al*, 2011). The increase in 2-AG following repeated stress is transient. We have demonstrated that, on the tenth day of exposure to restraint stress, 2-AG content in the amygdala is increased 20 min following stress onset and has returned to baseline by 60 min (Patel *et al*, 2009), where it remains at basal levels 24 h after the final stress (Hill *et al*, 2010b). These data would suggest that reductions in MAGL-mediated 2-AG hydrolysis likely account for the enhanced capacity of 2-AG signaling under conditions of chronic homotypic stress.

As with AEA, the effects of heterotypic stress on 2-AG content are less consistent. The only reliable finding to date is a reduction in 2-AG content within the hippocampus following CUS (Hill *et al*, 2005b; Lomazzo *et al*, 2015; Zhong *et al*, 2014). Asides from this, no studies have found any consistent changes in 2-AG content from chronic heterotypic stressors in any structures analyzed (Bortolato *et al*, 2007; Hill *et al*, 2005b, 2008b; Lomazzo *et al*, 2015; Wang *et al*, 2010). This variability may be a consequence of different time point for tissue collection after the final stress.

Taken together, these studies demonstrate a bidirectional effect of stress on the eCB system. In general, stress exposure reduces AEA and increases 2-AG levels throughout most brain regions examined. These effects appear to become amplified following chronic exposure to the same stressor, although chronic exposure to varying stressors seems to evoke less reliable changes in either of these molecules. Increases in CRH seem to be primarily responsible for the increased activity of FAAH and reduced levels of AEA within the amygdala, whereas elevations in corticosterone appear to be the primary mechanism by which stress increases 2-AG levels, at least within the mPFC and hippocampus (Figure 2).

Effects of Acute and Chronic Stress on CB1 Receptors

Few studies have examined the effects of acute stress on CB1 receptor levels. One report, looking at the binding site density of CB1 receptors in the amygdala, reported no effect of acute stress (Hill *et al*, 2009c). With the exception of the

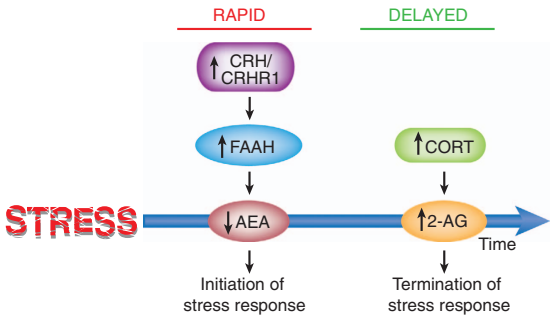

Figure 2. Temporal dynamics of anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) changes following stress exposure. Acute exposure to stress rapidly increases corticotropin-releasing hormone (CRH) signaling in the basolateral amygdala (BLA). The subsequent activation of CRHR1 receptors rapidly increases the enzymatic activity of fatty acid amide hydrolase (FAAH), resulting in a decrease of the inhibitory tone of AEA, which in turn contributes to the activation of hypothalamic–pituitary–adrenal (HPA) axis and stress-related behavioral responses. The delayed increase of brain corticosterone levels stimulates the release of 2-AG in the medial prefrontal cortex (mPFC) and paraventricular nucleus of hypothalamus (PVN). This increased 2-AG signaling at CB1 receptors contributes to negative-feedback inhibition of the HPA axis and termination of stress response.

PFC, chronic stress is associated with a downregulation of CB1 receptor expression. Using electrophysiology, mRNA, protein, and radioligand binding, downregulation of the CB1 receptor has been found in the hippocampus (Hill *et al*, 2005b, 2008b; Hu *et al*, 2011; Lee and Hill, 2013; Reich *et al*, 2009), hypothalamus (Hill *et al*, 2008b; Wamsteeker Cusulin *et al*, 2014; Wamsteeker *et al*, 2010), striatum (Hill *et al*, 2008b; Rossi *et al*, 2008, 2010; Wang *et al*, 2010), and dorsal root ganglion (Hong *et al*, 2011) following exposure to an array of both chronic homotypic and heterotypic stressors. The amygdala appears to be somewhat resistant to these effects as many reports have demonstrated no effect of chronic stress on CB1 expression using radioligand binding (Hill *et al*, 2008b; Lee and Hill, 2013); at the electrophysiological level, however, chronic homotypic stress has been shown to desensitize CB1 receptors in the BLA, at least on GABAergic terminals (Patel *et al*, 2009). As with many of the central effects of stress, several reports have demonstrated that this downregulation of CB1 receptor expression or signaling is reversed following a period of recovery (Lee and Hill, 2013; Rossi *et al*, 2008; Wamsteeker *et al*, 2010). There is strong evidence that these effects are mediated by corticosterone as chronic administration of corticosterone can recapitulate the stress-induced downregulation of the CB1 receptor (Bowles *et al*, 2012; Hill *et al*, 2008a; Hong *et al*, 2011; Rossi *et al*, 2008); additionally, blockade of the glucocorticoid receptor abrogates the effects of stress on the CB1 receptor (Hong *et al*, 2011; Rossi *et al*, 2008; Wamsteeker *et al*, 2010).

As mentioned, the mPFC is one region in the brain where both chronic homotypic and heterotypic stressors have reliably been found to increase the expression of the CB1 receptor at the mRNA-, protein-, and receptor-binding level (Bortolato *et al*, 2007; Hill *et al*, 2008b; Lee and Hill, 2013; McLaughlin *et al*, 2013; Zoppi *et al*, 2011). The mechanism responsible for the discrepancy in the mPFC (in comparison to other brain regions) is not clear but should be a topic of further research.

Taken together, this vast body of data present several common themes that could be relevant to stress neurobiology and will be the focus of the following sections. First, acute exposure to stress results in a relatively rapid decline of AEA signaling within the amygdala and hippocampus, which is likely mediated by an increase in CRH signaling at the CRHR1. The subsequent release of corticosterone following stress results in a delayed elevation in 2-AG, and possibly AEA, through several corticolimbic structures. Second, in response to chronic homotypic stress, there is a clear depletion of AEA and an elevation of 2-AG throughout the forebrain. These effects mirror what is seen with acute stress but appear to be amplified. Interestingly, chronic heterotypic stress exerts less reliable changes in either AEA or 2-AG. It is unclear whether this is due to differences in measurement of time points following the cessation of stress, the stress induction protocols used, or different brain areas examined. Finally, regardless of the modality, both chronic homotypic and heterotypic stress cause a significant decrease in CB1 receptor expression throughout all subcortical structures examined, but an increase in CB1 receptor expression in the mPFC. Collectively, these data indicate that the eCB system is exquisitely sensitive to stress exposure and exhibits dynamic and temporally specific changes in response to stressful stimuli.

Functional Role of eCB Signaling in the Neurobiological Effects of Stress

Role of eCBs in stress-induced regulation of the HPA axis. There is substantial evidence that eCB signaling regulates the HPA axis and this topic has been addressed in significant depth in previous reviews (Hill and Tasker, 2012; Steiner and Wotjak, 2008). As mentioned above, disruption of eCB signaling recapitulates many of the effects of stress, including activation of the HPA axis; the interpretation of these data would suggest that there is an eCB tone that actively functions to constrain activation of the HPA axis. Specifically, acute administration of a CB1 receptor antagonist results in an increase in basal induction of c-fos within the PVN, and an elevation in circulating levels of ACTH and/or corticosterone (Atkinson *et al*, 2010; Doyon *et al*, 2006; Ginsberg *et al*, 2010; Hill *et al*, 2010b; Manzanares *et al*, 1999; Newsom *et al*, 2012; Patel *et al*, 2004; Roberts *et al*, 2014; Steiner *et al*, 2008; Wade *et al*, 2006). The neuroanatomical site of action for this 'gatekeeper' role of eCB signaling on HPA output suggests an important role for the BLA. Specifically, local microinjections of CB1 receptor antagonists into the PVN (Evanson *et al*, 2010) or the mPFC (Hill *et al*, 2011b) have found no effect on basal corticosterone levels, but local administration of a CB1 receptor antagonist into the BLA is sufficient to increase HPA axis

activity (Ganon-Elazar and Akirav, 2009; Hill *et al*, 2009c). This suggests that the BLA may be an important site for eCB-mediated control of the HPA axis, although it is likely that there are potentially multiple redundant sites whereby eCB signaling exerts some effect on HPA axis function.

Interestingly, as described above, exposure to stress causes a rapid, CRH-mediated reduction in AEA content within the amygdala (Gray *et al*, 2015; Hill *et al*, 2009c; Patel *et al*, 2005b; Rademacher *et al*, 2008). As AEA is believed to represent the 'tonic' signal of the HPA axis, these data bring about the intriguing hypothesis that, under steady-state conditions, there is an AEA tone within the BLA, and possibly other areas, that functions to constrain the HPA axis under non-stressful conditions, and in response to stress this tone is disrupted, facilitating stress-induced activation of the HPA axis. Consistent with this hypothesis, acute administration of a CB1 receptor antagonist evokes a limited and specific pattern of neuronal activation in the brain, of which the BLA is one of the most prominent sites of activity, in addition to the PVN (Newsom *et al*, 2012; Patel *et al*, 2005a; Singh *et al*, 2004). More so, global or intra-BLA inhibition of AEA-hydrolysis dampens stress-induced activation of the HPA axis (Bedse *et al*, 2014; Hill *et al*, 2009c; Patel *et al*, 2004). In fact, the ability of systemic administration of a FAAH inhibitor to dampen neuronal activation within the PVN is completely reversible by local CB1 receptor antagonism within the BLA (Bedse *et al*, 2014), indicating that elevations of AEA signaling within the BLA are capable of attenuating stress-induced activation of the HPA axis, likely through a trans-synaptic relay from the BLA to the PVN. As such, these data would indicate that AEA is a regulatory signal within the BLA that suppresses activation of the HPA axis by gating BLA to PVN communication. Additionally, the rapid loss of AEA signaling within the BLA, through a CRHR1-mediated induction of FAAH activity, may represent an endogenous mechanism that regulates the magnitude of the HPA axis response to stress (Gray *et al*, 2015). Collectively, these data indicate that the BLA is an important modulatory seat for the effects of AEA signaling on the initiation of stress-induced activation of the HPA axis.

In addition to this gatekeeper role for the initial activation of the HPA axis in response to stress, there is also substantial evidence that an increase in 'phasic' eCB signaling contributes to limiting the magnitude and promoting the termination of stress-induced HPA axis activity. For example, acute administration of a CB1 receptor antagonist enhances neuronal activation within the PVN in response to stress and potentiates the magnitude and duration of stress-induced corticosterone secretion (Hill *et al*, 2011b; Newsom *et al*, 2012; Patel *et al*, 2004; Roberts *et al*, 2014). As 2-AG is believed to represent the 'phasic' signal of the eCB system, these data would suggest that stress-induced elevations in 2-AG content in the mPFC and hypothalamus (Evanson *et al*, 2010; Hill *et al*, 2011b), which are known to be important for glucocorticoid negative feedback on the HPA axis (Dallman, 2005; Diorio *et al*, 1993), contribute to termination of the stress response. Specifically, local administration of a CB1 receptor antagonist into the PVN impairs glucocorticoid-mediated rapid feedback inhibition of the HPA axis (Evanson *et al*, 2010), while blockade of CB1 receptors within the mPFC impairs normative recovery of the HPA axis following cessation of stress (Hill *et al*, 2011b). These findings are consistent with the ability of glucocorticoids to elevate eCB content (Atsak *et al*, 2012a; Di *et al*, 2005; Hill *et al*, 2010a) and suggest that eCB signaling is a necessary component of glucocorticoid-mediated negative feedback in the brain. As glucocorticoid receptors are predominantly localized in the somatodendritic region of postsynaptic cells (Johnson *et al*, 2005; Liposits and Bohn, 1993), our current working hypothesis is that glucocorticoids mobilize 2-AG to suppress excitatory inputs activated by exposure to stress and facilitate recovery to basal levels of activity.

In addition to their role in the termination of acute stress-induced activation of the HPA axis, there is also evidence that the progressive recruitment of 2-AG signaling during chronic homotypic stress contributes to adaptation of the HPA axis. Specifically, in adults, repeated exposure to the same stressor results in habituation of the HPA axis response, such that the magnitude of corticosterone release to stress exposure decreases over time (Grissom and Bhatnagar, 2009). As discussed above, this same stress regimen that promotes HPA axis habituation concomitantly results in elevations in 2-AG content throughout the forebrain (Dubreucq *et al*, 2012; Hill *et al*, 2010b; Patel *et al*, 2005b). Interestingly, acute administration of a CB1 receptor antagonist to animals before the final presentation of a repeated stressor effectively 'dishabituates' the animals such that dramatic elevations in neuronal activation throughout stress regulatory circuits are observed, such as in the BLA and PVN, and significant increases in corticosterone occur, relative to habituated animals (Hill *et al*, 2010b; Patel *et al*, 2005b). These data have led to the hypothetical model that eCB signaling is essential for stress adaptation and that the active recruitment of 2-AG signaling during repeated stress dampens neural circuits activated by stress, thereby resulting in a systems-level habituation to stress (Hill *et al*, 2010b; Patel and Hillard, 2008).

Collectively, these data highlight several important roles of the eCB system in the regulation of the HPA axis. First, tonic AEA signaling within the BLA gates HPA axis activity, and disruption of this AEA tone by stress (or through pharmacological/genetic means) results in the activation of the HPA axis and the secretion of corticosterone. Second, the increased levels of corticosterone following the launch of the stress response enhance 2-AG signaling in the mPFC and PVN; this may contribute to negative-feedback inhibition of the HPA axis and termination of the stress response. Third, upon repeated presentation of a common stressor, 2-AG levels are progressively enhanced within forebrain circuits and may mediate adaptation to stress and habituation of the HPA axis. As such, the eCB system is both a regulator and a target of stress-induced activation of the HPA axis.

Role of eCBs in stress-induced anxiety. A central theme that has emerged over the past decade is that eCBs have a

prominent role in the regulation of behavioral processes during times of stress or environmental challenge. Similarly to the regulation of HPA axis function described above, eCBs appear to reduce behavioral signs of anxiety specifically under stressful, aversive, or environmentally challenging conditions. For example, while pharmacological or genetic disruption of CB1 receptor signaling can moderately increase anxiety under basal conditions (Haller *et al*, 2002, 2004; Hill *et al*, 2011a; Martin *et al*, 2002; Mikics *et al*, 2009; Uriguen *et al*, 2004), this manipulation dramatically enhances anxiety induced by stress or environmental aversiveness (Gamble-George *et al*, 2013; Haller *et al*, 2004; Hill *et al*, 2011a). These data would suggest that dynamic changes in eCB signaling in response to stress may contribute to fluctuations in anxiety-like behavior, and subsequent work targeting AEA and 2-AG signaling independently has helped shed light on the potential mechanisms by which this process occurs.

The putative role of AEA in regulating anxiety was first highlighted by the Piomelli laboratory, who demonstrated that inhibition of AEA-hydrolysis by FAAH resulted in a reduction of anxiety (Kathuria *et al*, 2003). This ability of AEA signaling to reduce anxiety was then determined to be highly specific to the stressful nature of the environmental context such that inhibition of FAAH, through both pharmacological and genetic means, is more effective at reducing anxiety-related behaviors under challenging environmental conditions or after overt stressor exposure (Bluett *et al*, 2014; Dincheva *et al*, 2015; Haller *et al*, 2009; Hill *et al*, 2013b; Naidu *et al*, 2007). One potential interpretation of these data is that stress or aversive experiences produce anxiety through a rapid reduction in AEA signaling, which results in a transient decline in CB1 receptor signaling that facilitates the emergence of an anxiety state. These data are consistent with the aforementioned effects of CB1 receptor blockade on anxiety, as well as the fact that stress can rapidly reduce AEA content in anxiety-regulating circuits, such as the amygdala and hippocampus. Consistent with this model, we have demonstrated that stress-induced release of CRH rapidly triggers FAAH activity in the BLA to reduce AEA signaling, which in turn promotes the generation of anxiety (Gray *et al*, 2015). Importantly, central AEA levels are negatively correlated with anxiety-like behaviors (Bluett *et al*, 2014), and elevating AEA signaling can effectively curb anxiety induced by both acute and chronic stress (Bluett *et al*, 2014; Campos *et al*, 2010; Hill *et al*, 2013b; Lomazzo *et al*, 2015; Rossi *et al*, 2010). As such, it has been proposed that AEA may function as a mediator of 'emotional homeostasis' (Haller *et al*, 2013; Marco and Viveros, 2009), functioning to keep anxiety at bay in resting conditions, from which disruption of this signal by stress could contribute to the generation of an anxious state (Gunduz-Cinar *et al*, 2013a). There is evidence indicating that the neuroanatomical circuits mediating these effects are likely broad and diffuse. The ability of CRH signaling to trigger FAAH activity in the BLA has been explicitly linked to the generation of anxiety (Gray *et al*, 2015), but local overexpression of FAAH within the mPFC (creating a state of AEA deficiency) has also been found to be sufficient to induce anxiety, suggesting that this site may also be an important seat of the effects of AEA on anxiety control (Rubino *et al*, 2008). Finally, an additional report has found that administration of the AEA transport and metabolism inhibitor AM404 into the ventral hippocampus can reverse stress-induced anxiety (Campos *et al*, 2010). Given that brain-wide reductions in AEA correlate with anxiety following stress (Bluett *et al*, 2014), it is quite likely that while dynamic changes in AEA signaling within a discrete region may be capable of modulating anxiety, a systems-level shift in AEA signaling across many forebrain structures is likely relevant for contributing to stress-induced anxiety.

More recently, a role for 2-AG signaling in the regulation of anxiety has been examined using genetic and pharmacological approaches. Similar to the effects seen with CB1 receptor blockade, treatment with a MAGL inhibitor (to inhibit 2-AG metabolism) reduces basal anxiety in some studies but seems to particularly reduce anxiety under high-stress testing conditions (Aliczki *et al*, 2012, 2013; Busquets-Garcia *et al*, 2011; Kinsey *et al*, 2011; Sciolino *et al*, 2011). Given that stress can increase 2-AG release, one interpretation of these data would be that the mobilization of 2-AG acts to buffer against stress-induced anxiety and that augmentation of this signal through the inhibition of MAGL potentiates this effect. Consistent with this, central genetic 2-AG deficiency results in increased anxiety that can be reversed by acute normalization of 2-AG levels (Shonesy *et al*, 2014). There is also evidence that potentiation of 2-AG signaling can dampen anxiety induced by chronic stress (Sumislawski *et al*, 2011; Zhang *et al*, 2015; but see Lomazzo *et al*, 2015 for negative findings). These studies clearly support the notion that 2-AG also has a key role in regulating anxiety and stress responsivity. Interestingly, unlike AEA where these effects are all CB1 receptor dependent, in the case of 2-AG augmentation, both CB1 and CB2 receptors have been implicated; however, evidence supporting a CB1-dependent effect are stronger (Busquets-Garcia *et al*, 2011; Kinsey *et al*, 2011; Sciolino *et al*, 2011; Sumislawski *et al*, 2011).

Collectively, these data indicate that elevating both AEA and 2-AG signaling attenuates stress-induced anxiety, although the mechanisms of these effects may be different, especially as their dynamic regulation by stress occurs in a bidirectional manner. Although site-specific studies have identified the amygdala, mPFC, and hippocampus as potential substrates for the effects of AEA signaling on stress-induced anxiety, no work to date has identified the circuits through which 2-AG exerts its effects, although it seems reasonable to predict that overlapping populations of CB1 receptors are involved in both of these processes. Consistent with this hypothesis, emerging data strongly implicate CB1 receptors expressed on forebrain glutamatergic neurons as critical for the anxiolytic effects of eCBs, while CB1 receptors on GABAergic terminals do not appear to be involved in these anxiolytic effects (Rey *et al*, 2012; Rossi

et al, 2010; Ruehle *et al*, 2013). Additionally, a recent report has also indicated that CB1 receptors on serotonergic neurons may also be important for the regulation of anxiety (Dubreucq *et al*, 2012). Future work will require combined genetic and neuroanatomical approaches to clearly define the circuits by which eCB signaling regulates stress-induced anxiety.

Role of eCBs in stress-induced modulation of memory function. Stress- and emotional arousal-activated neurobiological systems have a highly adaptive role in ensuring that the strength of our memories will reflect their emotional significance (McGaugh, 2015). Although discussed in greater depth in these comprehensive reviews (Joels *et al*, 2011; Rodrigues *et al*, 2009; Roozendaal *et al*, 2009), in brief, stress and glucocorticoids typically facilitate learning performances (Akirav *et al*, 2004; Salehi *et al*, 2010) and enhance memory consolidation (de Kloet *et al*, 1999; Joels and Baram, 2009; Oitzl and de Kloet, 1992; Roozendaal, 2000; Sandi and Rose, 1994). Particularly, the BLA appears to have a crucial role in mediating the glucocorticoid-enhancing effects on memory consolidation (Roozendaal and McGaugh, 1996,1997). In contrast, stress and glucocorticoids impair memory retrieval during emotionally arousing tests both in animals (de Quervain *et al*, 1998) and human subjects (Bentz *et al*, 2013; de Quervain *et al*, 2009). In this section, we will discuss basic research findings of how the eCB system modulates stress effects on memory, although these have been reviewed in greater depth elsewhere (Atsak *et al*, 2012b; Morena and Campolongo, 2014a). Similar to what has been found with anxiety, the degree of stressfulness or aversiveness seems to contribute to the ability of eCB signaling to influence memory processes. With respect to memory acquisition, Campolongo *et al* (2012) showed that systemic injection of the AEA transport inhibitor AM404 impaired the acquisition of the novel object recognition task only in rats tested under higher stressful condition (ie, not handled and tested under bright light in an empty arena), without altering memory performance of rats tested under lower stressful condition (ie, extensively handled and tested under dim red light in an arena with familiar bedding (Campolongo *et al*, 2012).

However, local infusions into discrete brain regions have given different results. Tan *et al* (2011) reported that blockade of CB1 receptor activity in the BLA prevented the acquisition of associative fear memory when an olfactory cue was associated with a suprathreshold footshock intensity (0.8 mA) in a fear conditioning paradigm (Tan *et al*, 2011). Further, intra-BLA administration of AM404 strongly potentiated fear memory acquisition when the behavioral task was performed with a lower subthreshold footshock intensity (0.4 mA) that did not produce an associative freezing response in control rats at testing (Tan *et al*, 2011). These data would suggest that eCB signaling becomes recruited during highly aversive situations and under these conditions may act to impair or potentiate memory acquisition depending on the nature and intensity of the environmental stimuli, on the type of memory, and brain region involved.

Interestingly, with respect to memory consolidation, it appears more so that eCB signaling is important for the facilitation of consolidation that occurs in highly arousing situations. For example, intra-BLA blockade of eCB transmission dose-dependently disrupts memory consolidation in an inhibitory avoidance task (Campolongo *et al*, 2009). Consistent with this finding, others have reported that infusion of the CB1 receptor antagonist AM251 into the amygdala (Bucherelli *et al*, 2006) or hippocampus (de Oliveira Alvares *et al*, 2005) disrupts the consolidation of long-term memory, possibly by inhibiting long-term potentiation (LTP; de Oliveira Alvares *et al*, 2006). Again, it is likely that the level of stress elicited by the behavioral task results in differential engagement of eCBs to modulate memory consolidation. In support of this view are observations that rats trained in an inhibitory avoidance task with a higher footshock intensity (0.45 mA) present better memory retention than rats trained with a lower footshock intensity (0.35 mA), and this effect is paralleled by an increase in AEA levels within the amygdala, hippocampus, and mPFC (Morena *et al*, 2014b). Further, AEA has a crucial role in potentiating memory consolidation via CB1 receptor activation in these cortico-limbic circuits (Morena *et al*, 2014b). Similarly, de Oliveira Alvares *et al* (2010) found that a strong emotionally arousing experience is a necessary condition for the involvement of the hippocampal eCB system on memory consolidation. In this study, intra-hippocampal infusion of AM251 impaired the consolidation of a strong conditioning training (0.7 mA footshock intensity), but it did not induce any effect on a weak fear conditioning paradigm (0.3 mA footshock intensity; (de Oliveira Alvares *et al*, 2010). Thus it appears that a certain degree of emotional arousal leads to an optimal activation of stress mediators that is necessary for the eCB system to mediate the formation of a strong memory trace. Unlike what was seen with more moderate stressors, such as restraint, where AEA becomes reduced, in these highly arousing situations there actually appears to be an increase in AEA, which then contributes to the consolidation of these emotionally salient memories.

The importance of the eCB system in facilitating the extinction of emotionally aversive memories has also been consistently reported by several groups. Over a decade ago, Marsicano *et al* (2002) and subsequent investigators demonstrated that genetic deletion of CB1 receptors, as well as systemic or intra-BLA inhibition of eCB transmission, robustly inhibits fear extinction (Chhatwal *et al*, 2005; Ganon-Elazar and Akirav, 2009; Marsicano *et al*, 2002; Suzuki *et al*, 2004). Tone presentation during extinction trials results in elevated levels of eCBs in the BLA (Gunduz-Cinar *et al*, 2013b; Marsicano *et al*, 2002). Chhatwal *et al* (2005) and others demonstrated that intraperitoneal or intracerebroventricular injection of AM404 enhances fear extinction via a CB1-dependent mechanism (Bitencourt *et al*, 2008; Chhatwal *et al*, 2005; Pamplona *et al*, 2008). A more recent study underlined the importance of the BLA in these AEA

facilitating effects on memory extinction. Gunduz-Cinar *et al*, 2013b found that intra-BLA infusions of the FAAH inhibitor AM3506 potentiate memory extinction through the activation of CB1 receptors (Gunduz-Cinar *et al*, 2013b). Thus the evidence obtained to date strongly suggests that the activation of the eCB system represents an important step for the facilitation of emotionally aversive memory extinction to occur (Gunduz-Cinar *et al*, 2013a).

It has become apparent that glucocorticoids are an important mechanism of how the eCB system becomes engaged by stress to modulate memory processes. As mentioned, glucocorticoid administration selectively enhances the consolidation of emotionally arousing experiences within the BLA (Roozendaal and McGaugh, 1996, 1997). Campolongo *et al* (2009) provided the first evidence that this mechanism is mediated by the amygdalar eCB system. They found that intra-BLA administration of a non-impairing dose of the CB1 receptor antagonist AM251 after inhibitory avoidance training blocked the memory-enhancing effects induced by systemic injection of corticosterone, suggesting that glucocorticoids recruit eCB signaling within the BLA to enhance memory consolidation (Campolongo *et al*, 2009). Similarly, a separate study reported that a CB1 receptor antagonist infused into the hippocampus blocked the memory enhancement induced by the synthetic glucocorticoid dexamethasone (de Oliveira Alvares *et al*, 2010). Atsak *et al* (2014) have recently shown that these glucocorticoid effects on the eCB system, within the BLA, are dependent on a membrane glucocorticoid receptor, thus involving a rapid non-genomic mechanism (Atsak *et al*, 2014). Furthermore, they found that eCB signaling was also required for glucocorticoids to induce an increase in CREB phosphorylation in BLA pyramidal neurons (Atsak *et al*, 2014). A similar model has also been proposed for glucocorticoid-impairing effects on memory retrieval. Indeed, systemic administration of corticosterone 1 h before retention testing, by increasing hippocampal 2-AG levels, impairs the retrieval of a contextual fear memory; an effect which is reversible by pharmacological blockade of hippocampal CB1 receptors (Atsak *et al*, 2012a). In line with these findings, the ability of glucocorticoids to enhance the extinction of emotionally aversive memories is also dependent on CB1 receptors and can be replicated by administration of AM404 (Bitencourt *et al*, 2014). These effects are all consistent with data indicating that glucocorticoids increase AEA and/or 2-AG content within the amygdala and hippocampus (Atsak *et al*, 2012a; Hill *et al*, 2010a). Mechanistically, these data would suggest that eCB signaling is downstream of glucocorticoids, and more so eCB signaling mediates the effects of glucocorticoids on the modulation of memory processes. Interestingly, at least with respect to consolidation, the role of eCB signaling, while downstream of glucocorticoids, is upstream of norepinephrine, as modulation of eCB signaling did not block the effects of beta-adrenoreceptor activation on enhancing memory consolidation (Atsak *et al*, 2014). More so, FAAH inhibition has been found to increase norepinephrine levels within the amygdala in response to stress (Bedse *et al*, 2014), suggesting that the effects of eCB signaling are upstream of norepinephrine release. Collectively, these findings suggest that glucocorticoids rapidly recruit eCBs in the BLA to increase the sensitivity of pyramidal neurons to the memory-enhancing effects of norepinephrine (McIntyre *et al*, 2002; Quirarte *et al*, 1997). The evidence summarized above clearly indicates that the eCB system modulates cognitive function in a manner dependent on the aversiveness of the environmental condition and on the degree of emotional arousal at the time of testing.

Role of eCBs in stress-induced regulation of pain. With respect to acute stress, there is evidence that an increase in eCB signaling contributes to non-opioid stress-induced analgesia (Hu *et al*, 2014). The initial demonstration of this effect found that CB1 receptor-deficient mice did not exhibit antinociception following exposure to swim stress (Valverde *et al*, 2000). Following on this finding, Hohmann *et al* (2005) presented a highly comprehensive characterization of the role of eCB signaling in non-opioid stress-induced analgesia. Specifically, they demonstrated that exposure to footshock elevated both AEA and 2-AG within the periaqueductal gray and that blockade of CB1 receptors in this region prevented footshock stress-induced analgesia (Hohmann *et al*, 2005). These results have been extended by reports demonstrating that analgesia occurring after exposure to the context of footshock similarly involves an eCB mechanism in the periaqueductal gray (Olango *et al*, 2012). More so, these results have been extended by other reports that global disruption of CB1 receptor signaling block stress-induced analgesia (Kurrikoff *et al*, 2008), as well as a recent report that eCB signaling similarly mediates stress-induced analgesia in fish (Wolkers *et al*, 2015). As such, there seems to be substantial evidence that stress-induced increases in eCB signaling mediate the acute analgesic effect that occurs after exposure to stress. In addition to these effects, one recent report demonstrated that chronic inhibition of FAAH, but not MAGL, is capable of reversing the development of hyperalgesia after exposure to chronic stress (Lomazzo *et al*, 2015), suggesting the possibility that reductions in AEA signaling, which occur following chronic stress, may contribute to the development of hyperalgesia. As such, the current data do suggest that dynamic changes in eCB signaling from acute and chronic stress may differentially contribute to changes in pain sensitivity.

Role of eCBs in stress-induced regulation of reward. There are two domains within reward processes in which changes in eCB signaling from stress could be relevant. First, stress exposure is known to be a precipitating factor in drug use and relapse (Koob *et al*, 2014). With respect to eCB signaling, there are data that suggest the possibility that eCB signaling may be involved in this process. For example, CB1 receptor-deficient mice have been found to be resistant to elevations in alcohol consumption in response to footshock (Racz *et al*, 2003); however, acute CB1 receptor antagonism did not

prevent alcohol reinstatement in response to footshock stress (Economidou *et al*, 2006). Without further evidence to help resolve this discrepancy, one interpretation could be that eCB signaling contributes to stress-induced alcohol consumption but not stress-induced relapse following cessation. Similar discrepancies have been found with cocaine whereby CB1 receptor antagonism does not block footshock-induced reinstatement of cocaine-seeking behavior (De Vries *et al*, 2001; Kupferschmidt *et al*, 2012) but has been found to reverse the reinstatement of cocaine-seeking behavior in response to swim stress (Vaughn *et al*, 2012) or direct administration of CRH (Kupferschmidt *et al*, 2012). Here it is possible that the stress modality itself could have differential effects on eCB signaling (as discussed above) in which eCB signaling could be important for some forms of stress-induced cocaine reinstatement, while it is dispensable for others. More work, particularly in the context of discrete neural circuit manipulations, is required to fully understand the contributions eCB signaling has with respect to stress-induced drug use and relapse.

The second domain of reward processing where the eCB system may be relevant for the effects of stress is the development of impairments to reward sensitivity (or 'anhedonia') that develop following exposure to chronic stress. Specifically, animal studies have reliably demonstrated that exposure to chronic stress can produce anhedonia, as gauged by the preference or consumption of sucrose. Interestingly, chronic administration of a FAAH inhibitor during chronic stress has been shown to counter the development of anhedonia (Bortolato *et al*, 2007; Rademacher and Hillard, 2007), suggesting that the deficiency in AEA signaling that emerges from chronic stress may render reward circuits less sensitive and promote the development of anhedonia. Alternately, both genetic (Martin *et al*, 2002) or pharmacological (Rademacher and Hillard, 2007) disruption of CB1 receptor signaling can significantly potentiate and accelerate the development of anhedonia in response to chronic stress. This would suggest that potential increases in 2-AG signaling that occur following chronic stress could be a protective mechanism that is elevating in an attempt to buffer against the development of anhedonia following chronic stress. Consistent with this, it has also been found that chronic administration of a MAGL inhibitor during chronic stress can counter the development of anhedonia (Zhong *et al*, 2014). Very reminiscent of what is seen following exposure to stress, chronic intermittent alcohol consumption increases striatal 2-AG levels leading to a compensatory reduction of CB1 receptor functionality and a loss of CB1-mediated synaptic plasticity in the lateral part of the dorsal striatum (DePoy *et al*, 2013). Future work is required to determine whether this rearrangement of the eCB system by chronic alcohol contributes to changes in emotional behavior or stress sensitivity.

Role of eCBs in stress-induced synaptic plasticity. A common theme that has emerged from this review is that eCB signaling is recruited or modulated by stress mediators, such as glucocorticoids or CRH, to modulate a physiological output, such as anxiety or memory. At a mechanistic level, eCBs likely modulate these processes by impacting a range of eCB-mediated synaptic plasticity mechanisms. Retrograde eCB signaling mediates multiple forms of synaptic plasticity, such as long-term depression (LTD) or depolarization-induced suppression of inhibition/excitation (DSI/E). Recently, the effects of stress on synaptic transmission and plasticity have become a growing area of research (reviewed in (Joels *et al*, 2009; Popoli *et al*, 2011; Senst and Bains, 2014; Bains *et al*, 2015). As discussed, the CB1 receptor is expressed throughout the brain and is present on glutamate and GABA synapses in stress-sensitive circuits, such as the hypothalamus, mPFC, hippocampus, and amygdala. This includes CB1 receptor expression on inputs to critically stress-sensitive regions, including the BLA (Azad *et al*, 2003; Huang *et al*, 2003; Yoshida *et al*, 2011) and CeA (Kamprath *et al*, 2011; Ramikie *et al*, 2014; Roberto *et al*, 2010) neurons, which regulate motivational, affective, and autonomic responses to stress, as well as parvocellular neurosecretory cells (PNCs) in the PVN that regulate the release of CRH to promote activation of the HPA axis (Herkenham *et al*, 1990; Wittmann *et al*, 2007). The eCB system is extremely labile in response to stress and this has a profound impact on both short-term and long-term plasticity at synapses, particularly in the hypothalamus.

In the PVN, eCB synthesis can be driven by glucocorticoids; this results in a presynaptic decrease in glutamate release (Di *et al*, 2003; Wamsteeker *et al*, 2010), which contributes to dampening HPA axis activity following stress (Evanson *et al*, 2010). Why these effects are not observed at GABA synapses, which express CB1 receptors is something that requires further investigation, but does suggest a level of specificity with respect to which synapses are targeted by glucocorticoid–eCB interactions. Consistent with these *in vitro* effects, activation of the HPA axis *in vivo* can also impact eCB signaling in the hypothalamus. In response to acute stress, eCB signaling is positively modulated in a corticosterone-dependent manner as evidenced by an increase eCB signaling at both glutamate and GABA synapses (Wamsteeker *et al*, 2010). This phenomenon of glucocorticoid regulation of synaptic function through an eCB mechanism has been found to be a widespread phenomenon throughout most of the brain. Within the hippocampus (Wang *et al*, 2012b) and mPFC (Hill *et al*, 2011b), *in vitro* administration of corticosterone or *in vivo* exposure to stress can enhance eCB-mediated suppression of GABAergic transmission in a glucocorticoid receptor-dependent manner. Similarly, within the dorsal raphe, glucocorticoids can rapidly suppress glutamate release through an eCB mechanism; whether this mechanism also applies to GABAergic synapses remains to be explored (Wang *et al*, 2012a). The effects of glucocorticoids within the amygdala, however, seem to be different than what is seen in these other regions. In the BLA, both *in vivo* exposure to stress or *in vitro* application of corticosterone result in an increase, not a decrease, in glutamate currents through a

mineralocorticoid receptor-dependent process (Karst *et al*, 2010). However, the application of corticosterone to a BLA slice from an animal that has been exposed to stress results in a suppression of glutamate release, which is mediated by the release of eCBs (Karst *et al*, 2010). This would suggest that stress initially primes the BLA through an increase in excitatory transmission; once corticosterone is released in response to stress, it reduces excitation of the BLA, through an eCB mechanism, leading to an attenuation of amygdala activity. The temporal pattern of these processes mirrors the rapid decline (through CRH) and subsequent increase (through corticosterone) of AEA dynamics within the amygdala, suggesting the possibility that regulation of glutamatergic transmission could be the mechanism by which fluctuations in AEA contribute to the initiation and termination of stress-induced anxiety.

In addition to the importance of eCB signaling in mediating synaptic changes from glucocorticoids, there is also evidence that exposure to stress itself may actually influence the functionality of CB1 receptors. Specifically, studies examining the effects of CB1 stimulation in the BNST have shown that exposure to stress causes a switch in the effects on CB1 receptor stimulation on neuronal firing. Rather than promoting LTD, CB1 receptor activation after stress promotes LTP (Glangetas *et al*, 2013). Consistent with this switch in functionality, it has also been reported that the application of a CB1 receptor agonist to hippocampal slices from stressed animals switches CB1 receptor activation from suppressing glutamatergic transmission to enhancing LTP (Reich *et al*, 2013). The mechanisms behind this ability of CB1 signaling to switch from inhibitory to excitatory have not been explored but may explain the interactive effects of stress and cannabinoids on causing opposite responses to those typically seen in non-stressed animals (Fokos and Panagis, 2010; Hill and Gorzalka, 2004; Patel *et al*, 2005a).

As discussed previously, there is significant evidence that chronic exposure to stress compromises CB1 receptor expression and signaling at the synaptic level. For example, repeated homotypic stress causes a progressive loss of eCB signaling at both glutamate and GABA synapses in the PVN (Wamsteeker *et al*, 2010). Consistent with this phenomenon, chronic stress-induced decreases in CB1 receptor regulation of GABAergic transmission has also been reported in the striatum (Rossi *et al*, 2008), amygdala (Patel *et al*, 2009), and hippocampus (Hu *et al*, 2011), and a loss of CB1 regulation of glutamatergic transmission has been found in the nucleus accumbens (Wang *et al*, 2010) and dorsal raphe (Haj-Dahmane and Shen, 2014), suggesting that a loss of presynaptic CB1 receptor functionality may be a general feature in regions that gate stress responses. Consistent with this, eCB-mediated forms of synaptic plasticity have also been found to become impaired following chronic stress, such as DSE and LTD in the nucleus accumbens (Wang *et al*, 2010), as well as DSI (Zhong *et al*, 2014) and LTP in the hippocampus (Zhang *et al*, 2015). Quite surprisingly, the amygdala, again, seems to be the one brain region that shows different effects under conditions of chronic stress. Despite exhibiting desensitization of CB1 receptors on GABA terminals within the BLA following chronic stress (Patel *et al*, 2009), repeated exposure to restraint stress actually increases both DSI (Patel *et al*, 2009) and inhibitory LTD (Sumislawski *et al*, 2011) in the BLA. The interpretation of these data are that, despite desensitization of the CB1 receptor on GABAergic terminals, the enhancement of 2-AG signaling produced by chronic homotypic stress overcomes this reduced receptor sensitivity to produce enhanced forms of synaptic plasticity. It is not clear why the effects of enhanced 2-AG are only apparent within the amygdala but may relate to the potential sensitivity of 2-AG signaling in the amygdala to repeated stress (Hill *et al*, 2010b). These findings are not surprising in some regards, though, given that the amygdala has often been found to exhibit differential effects to chronic stress than many other brain structures (McEwen, 2012b).

The loss of CB1 receptors from chronic stress is likely due to activation of genomic glucocorticoid receptors that trigger a loss of functional presynaptic CB1 receptors, as the effect is blocked by the glucocorticoid receptor antagonist RU486 (Rossi *et al*, 2008; Wamsteeker *et al*, 2010). The mechanisms through which glucocorticoid receptor activation translates into a downregulation of CB1 receptors remain unresolved, although it is likely due to either direct negative regulation of the CB1 receptor gene by glucocorticoids (as has been demonstrated in the striatum; (Mailleux and Vanderhaeghen, 1993) or to glucocorticoid-mediated recruitment of eCB signaling and consequential agonist-induced receptor desensitization (as has been found after sustained elevation of 2-AG signaling; (Schlosburg *et al*, 2010).

As with most effects of chronic stress (McEwen, 2012b), the loss of eCB signaling in response to homotypic stress is not permanent and recovers passively in a few days (Rossi *et al*, 2008; Wamsteeker *et al*, 2010). This recovery, however, can be accelerated if, immediately after homotypic stress, the animal is exposed to a single novel stress (Wamsteeker Cusulin *et al*, 2014). We propose that stressor salience is the key determinant regulating the efficacy of eCB signaling in the PVN. Specifically, experiences with low salience such as homotypic or repetitive, predictable stress impair presynaptic CB1 receptor function. By contrast, these changes can be effectively reversed and the eCB system can be re-engaged by a novel stress that has a high relative salience. Importantly, these changes do not occur upstream of the PVN but are directly linked to changes in the local activity of synaptic inputs to PNCs as this effect is recapitulated by enhancing synaptic drive or neuronal activation in the PVN (Wamsteeker Cusulin *et al*, 2014). This is consistent with findings that ongoing synaptic activity tunes CB1 receptor function and, as a result, modulates the efficacy of eCB signaling (Chen *et al*, 2007; Chen *et al*, 2003). The necessity of synaptic activity for CB1 receptor function is further supported by evidence that the presynaptic activity state has a role in regulation of eCB inhibition of GABA release.

Specifically, increasing presynaptic firing rates in the hippocampus can recover inhibition of GABA release induced by a CB1 receptor agonist, in an N-type Ca^{2+}-channel-dependent manner (Foldy *et al*, 2006). A similar explanation may underlie observations that exercise can re-set CB1 receptor sensitivity in the striatum following stress (De Chiara *et al*, 2010). Future work is required to determine whether the lability of these chronic stress effects on CB1 receptor function are common throughout the brain or are specific to discrete synapses and circuits.

In addition to synaptic plasticity, there is also evidence that eCB signaling is important for the regulation of neurogenesis, another form of neuroplasticity. For example, administration of AM404 can reverse acute stress-induced suppression of cell proliferation in the dentate gyrus (Hill *et al*, 2006). Consistent with this, administration of the phytocannabinoid cannabidiol is capable of reversing chronic stress-induced reductions in neurogenesis and this appears to be driven by a cannabidiol-mediated inhibition of FAAH activity and consequential elevation in AEA levels (Campos *et al*, 2013). More so, inhibition of MAGL to elevate 2-AG levels during chronic stress similarly prevents impairments in hippocampal neurogenesis (Zhang *et al*, 2015). As previous work has shown that elevating AEA signaling and promoting CB1 receptor signaling is capable of promoting cell proliferation and neurogenesis (Aguado *et al*, 2005; Jiang *et al*, 2005), these findings imply that impairments in AEA and/or CB1 receptor signaling from chronic stress could result in reductions in neurogenesis.

The functional effects of the loss of eCB-mediated signaling and synaptic plasticity from chronic stress are not completely understood; however, it is increasingly appreciated that impairments in synaptic plasticity render a given neural circuit less flexible and adaptable and may represent a primary substrate underlying the transition of chronic stress into mental illness (Calabrese *et al*, 2009; McEwen, 2012a; Pittenger and Duman, 2008). Accordingly, chronic stress-induced collapse of eCB signaling may be involved in the development of allostatic load from chronic stress and contribute to the development of psychiatric conditions, such as depression. This hypothesis is consistent with findings that augmenting eCB signaling in the presence of chronic stress can stabilize and curb stress-induced changes in plasticity and produce potential antidepressant- or anxiolytic-like effects (Bortolato *et al*, 2007; Hill *et al*, 2013b; Lomazzo *et al*, 2015; Sumislawski *et al*, 2011; Zhang *et al*, 2015; Zhong *et al*, 2014).

Translational Studies in Humans

This body of research creates a compelling argument for the importance of eCB signaling in regulating and mediating multiple aspects of the stress response. Interestingly, experimental studies in human populations are generally consistent with those performed in rodents, and there is significant data indicating the importance of the eCB system to regulate stress and anxiety in humans as well (see Hillard *et al*, 2012 and Hill and Patel, 2013c for more in-depth reviews). For example, exposure of humans to the Trier social stress test has been found to elevate circulating levels of 2-AG (Hill *et al*, 2009d) or AEA (Dlugos *et al*, 2012), indicating that stress exposure in humans similarly elevates an eCB signal. Although it is not clear why AEA was found to elevate, instead of decrease, in response to stress, as is found in the rodent brain studies, however, it is worth noting that both chronic stress and corticosterone treatment are found to increase circulating levels of AEA despite reducing central AEA in rodents (Bowles *et al*, 2012, 2015; Hill *et al*, 2008a). Consistent with this, circulating AEA levels have been found to correlate with cortisol in humans (Hill *et al*, 2013a), suggesting that peripheral glucocorticoids may increase circulating AEA in humans. Interestingly, stress induction, through the use of personally relevant stress-related imagery, has been found to result in a progressive decline in the circulating levels of AEA, suggesting that some stressors may indeed reduce circulating AEA similar to what is seen in the rodent brain (Mangieri *et al*, 2009).

With respect to functionality, there is a surprisingly consistent body of literature implicating AEA signaling in humans in the regulation of anxiety and amygdala activity. For example, in both healthy and psychiatric populations, lower levels of circulating AEA have been found to correlate with higher anxiety (Dlugos *et al*, 2012; Hill *et al*, 2008c). Studies examining the effects of elevated AEA in humans have capitalized on the occurrence of a single-nucleotide polymorphism (SNP) in the *FAAH* gene that results in elevated levels of AEA (Sipe *et al*, 2010). Specifically, the substitution of an ancestrally conserved proline to a variant threonine (P129T or C385A) results in reduction in FAAH expression, possibly due to an increased vulnerability to proteolytic degradation (Chiang *et al*, 2004; Sipe *et al*, 2002). The recent development of a transgenic mouse possessing this C385A polymorphism in FAAH has verified that a similar reduction in FAAH expression and function, coupled to an increase in AEA levels within the brain, is seen in A carriers (Dincheva *et al*, 2015). Human carriers of the A allele of this SNP have been found to exhibit reduced activation of the amygdala in response to threat cues, accelerated habituation of the amygdala to threat cues, reduced trait anxiety, increased extinction of fear memories, and enhanced prefrontal–amygdala resting-state coupling (Dincheva *et al*, 2015; Gunduz-Cinar *et al*, 2013b; Hariri *et al*, 2009). Similarly, mouse studies of the A carriers also demonstrate that elevated AEA signaling is associated with reduced anxiety, accelerated fear extinction, increased innervation of prefrontal cortical inputs to the BLA, and reduced activation of the BLA in response to stress (Dincheva *et al*, 2015). One study has reported increased startle reactivity to emotional cues in A carriers (Conzelmann *et al*, 2012); however, this process could be functionally distinct from amygdala reactivity and anxiety. Together, these studies demonstrate that AEA signaling is likely an important regulator of anxiety in humans, and this

effect is likely mediated by dampened activity of the amygdala.

There is also evidence that eCB signaling in humans is also involved in the HPA axis response to stress. For example, when exposed to parabolic stress, individuals who exhibit an increase in circulating levels of 2-AG exhibit minor changes in HPA axis function, while those who exhibit no change in 2-AG levels possess massive increases in cortisol, indicating an inverse relationship between the ability of an individual to mobilize eCBs in response to stress and the magnitude of HPA axis activation (Chouker *et al*, 2010). One study has suggested that pharmacological blockade of the CB1 receptor in humans may directly increase cortisol levels, supporting the hypothesis that, similar to rodents, eCB signaling constrains activation of the HPA axis in humans (Goodwin *et al*, 2012).

The potential importance of the eCB system in regulating emotional behavior in humans was highlighted by the outcomes of the rimonabant trials in humans, where the effects of CB1 receptor blockade were tested in humans for the treatment of obesity. Several studies and meta-analyses demonstrated that indices of anxiety and depression significantly increased following sustained rimonabant treatment (Christensen *et al*, 2007; Hill and Gorzalka, 2009a; Mitchell and Morris, 2007; Topol *et al*, 2010); in fact, there was even a published case report of the *de novo* development of melancholic depression following the onset of rimonabant use (de Mattos Viana *et al*, 2009). Based on the described role of eCB signaling in the regulation of the neurobehavioral effects of stress, it is reasonable to hypothesize that sustained disruption of eCB signaling could result in the development of anxiety and depression by impairing the buffering effects of eCB signaling on the stress response, rendering the individual more vulnerable to the adverse effects of stress. Consistent with this, experimental studies have demonstrated that administration of rimonabant can directly potentiate stress-induced anxiety (Bergamaschi *et al*, 2014), promote negative memory bias (Horder *et al*, 2012), suppress positive memory recall (Horder *et al*, 2009), and dampen activation of reward circuits in the brain in response to pleasurable stimuli (Horder *et al*, 2010). Additionally, SNPs in the CB1 receptor gene *CNR1* are associated with increased vulnerability to develop depression and anhedonia following early life stress (Agrawal *et al*, 2012) or current life stress (Juhasz *et al*, 2009), as well as impaired neural responses to positive emotional stimuli and a heightened likelihood to exhibit antidepressant resistance (Domschke *et al*, 2008). Although it is not known whether any of these SNPs result in altered CB1 receptor expression or activity, it has been postulated that it could result in decreased mRNA stability and reduced CB1 receptor expression (Domschke *et al*, 2008). Taken together, these data demonstrate that impairments in eCB signaling in humans result in an increased sensitivity to the effects of stress, consistent with a stress-buffering role of eCB signaling in humans, and an enhanced vulnerability to develop stress-related psychiatric illnesses. In line with this, several studies have demonstrated that both depression and posttraumatic stress disorder (PTSD) are associated with reduced levels of circulating eCBs (Hill *et al*, 2008c, 2009d, 2013a; Neumeister *et al*, 2013).

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Collectively, this review provides a broad overview of the rapidly growing field of research investigating the neurobiological interactions between stress and the eCB system. From this analysis, there are several points to emphasize on what the current state of knowledge informs us of this relationship. First, exposure to stress, both acute and chronic, appears to generally result in a bidirectional regulation of the two eCB ligands AEA and 2-AG, with AEA being reduced from stress and 2-AG being increased from stress. The temporal nature of these changes seems to be distinct such that the reduction of AEA appears to occur relatively quickly in response to stress and is mediated by CRH activating the CRHR1 receptor to increase AEA hydrolysis by FAAH. This mechanism appears to be maintained under conditions of chronic stress where sustained exposure to glucocorticoids upregulates CRH signaling, which maintains long-lasting increases in FAAH and reductions in AEA. In the acute response to stress, this decline in AEA signaling seems to contribute to the manifestation of an anxiety state, the activation of the HPA axis, the impairment in fear extinction, and the suppression of cell proliferation in the neurogenic region of the hippocampus. Under conditions of chronic stress, the more sustained reduction in AEA seems to still contribute not only to all of the facets described above but also may have a role in the development of anhedonia and hyperalgesia. As such, inhibition of FAAH is able to reverse many of the effects of acute and repeated stress.

With respect to acute stress, the increase in 2-AG is delayed and seems to be mediated by increases in corticosterone from stress. The fact that 2-AG responses to stress are amplified under conditions of repeated exposure, which concomitantly results in habituation of corticosterone responses, suggests that a different mechanism contributes to this increase in 2-AG signaling. Ongoing research is seeking to determine what these mechanisms are, but a reduction in MAGL expression at the membrane appears to be one potential factor resulting in enhanced 2-AG signaling capacity after repeated stress (Sumislawski *et al*, 2011). The primary importance of the increase in 2-AG signaling in response to stress seems to be to buffer and constrain the effects of stress on the brain and facilitate termination of the stress response. Given the effects of CB1 receptor blockade during acute stress, it also seems likely that this increase in 2-AG may contribute to ability of acute stress to promote drug intake (at least alcohol intake), as well as contribute to stress-induced analgesia. At the synaptic level, the ability of glucocorticoids to mobilize 2-AG signaling also seems to be important for many of the shifts in synaptic plasticity that

take place in response to stress. Under conditions of chronic stress, not only does the progressive increase in 2-AG signaling seem important for stress adaptation and habituation to occur, but the elevated levels of 2-AG may also function to constrain many aspects of stress as blockade of the CB1 receptor results in an exacerbation of many of the effects of stress, such as anxiety and anhedonia. As such, there seems to be a clear ying–yang relationship for AEA and 2-AG, with both molecules ultimately providing a stress-inhibitory role, but the reduction in AEA signaling being relevant for the initiation and manifestation of the effects of stress, while the increase in 2-AG being relevant for tempering and terminating the stress response. These phenomena can be visualized in Figure 3. Moving forward, it will be important to identify the mechanisms by which these changes in eCB signaling occur, the circuit-specificity of these effects, and their impact on synaptic transmission. With the advent of many refined technologies that facilitate the examination of these questions, such as optogenetics or the recently described STORM super-resolution of the CB1 receptor (Dudok *et al*, 2015), we can hopefully begin to understand the complexity of these phenomena at both a synaptic level and a circuit level.

Another concept that merits further attention here is the potential relevance of the collapse of CB1 receptor signaling following chronic stress. Throughout this review, we have highlighted these data indicating the importance of eCB signaling in buffering against the effects of stress, thus it is not surprising that the impairments in this system that emerge following chronic stress seem to associate with the development of adverse responses, such as anxiety and anhedonia, which are classically linked with the onset of psychiatric conditions. In the past few decades, the concept of allostatic load has been championed by Bruce McEwen (Karatsoreos and McEwen, 2011), whereby it is hypothesized that the continual wear and tear from stress exposure results in an increased vulnerability to the adverse effects of stress. In this context, it is interesting to consider the possibility that a collapse of the eCB system could be a contributor to the effects of allostatic load. This is consistent with the fact that impairments in this system are associated with a greater vulnerability to stress-induced mental illnesses and that, in the animal models, augmentation of the eCB system can ward off the development of many of these adverse effects of chronic stress that are signs of allostatic load. Future work, both basic and clinical, should further investigate whether impairments in the eCB system are a potential contributor to allostatic load and, more so, if there are biomarkers in this system that could act as an index of stress fatigue or a predictive measure for vulnerability to psychiatric illness.

Based on these data, there has been a significant interest in therapeutics development around eCB augmenting agents for stress-related neuropsychiatric disorders (see (Gaetani *et al*, 2009; Hill *et al*, 2009b; Hill and Patel, 2013c; Neumeister *et al*, 2015). Results of pending clinical trials with FAAH inhibitors will reveal therapeutic potential of this

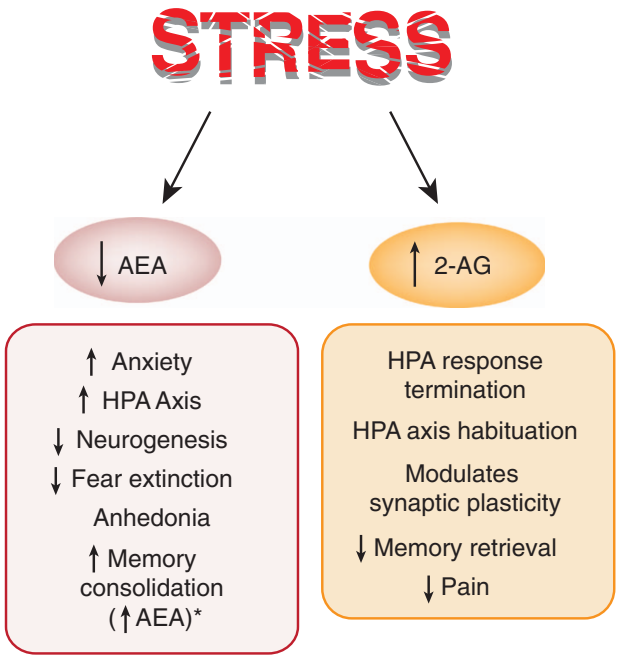

Figure 3. Schematic illustration of behavioral outputs regulated by the interaction between stress and endocannabinoids. Exposure to stress, both acute and chronic, generally results in a bidirectional regulation of anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), with AEA being reduced and 2-AG being increased from stress. Although the behavioral outputs regulated by AEA and 2-AG in some cases likely overlap, the decline in AEA signaling seems to mainly contribute not only to the manifestation of an anxiety state, the activation of the HPA axis, the suppression of neurogenesis in the hippocampus, and an impairment in fear extinction but also may have a role in the development of anhedonia and hyperalgesia. Unlike AEA, the behavioral influences of 2-AG are less characterized as selective tools for manipulating 2-AG signaling have only been recently developed. The stress-induced increase in 2-AG is believed to buffer and constrain the effects of stress on the brain, particularly by contributing to termination of stress-induced HPA axis activation and promoting habituation to stress, to possibly contribute to the ability of acute stress to shift synaptic plasticity, to mediate stress impairing effects on memory retrieval, and contribute to stress-induced analgesia. *Interestingly, with respect to memory consolidation, it appears that the stress/pain associated with the training procedure (ie, footshock exposure) leads to an increase in limbic AEA levels that, in turn, contributes to enhance aversive memory consolidation, distinguishing this effect from the other noted effects that are related to the decline in AEA signaling typically seen following stress.

class of drug for stress-related psychiatric disorder, particularly PTSD and possibly major depression. The potential utility of MAGL inhibitors could be more problematic, as unlike the fact that chronic elevations in AEA do not seem to modulate CB1 receptor function (Lichtman *et al*, 2002), chronic elevations in 2-AG signaling, through administration of high doses of MAGL inhibitors, seem to clearly desensitize the CB1 receptor (Schlosburg *et al*, 2010). As such, the utility of these drugs may be more limited, although additional studies have indicated that lower-dose titrations of MAGL inhibitors do not result in a desensitization of CB1 receptors (Kinsey *et al*, 2013). That being said, the possibility of alternate routes of interfering with eCB metabolism seems

like a possibility as well. For example, recent studies have identified an additional eCB inactivation pathway mediated by COX-2, which may regulate anxiety-related behaviors and stress responses (Hermanson *et al*, 2013). The recent development of substrate-selective inhibitors of COX-2 have allowed for the pharmacological dissection of COX-2-regulated eCB signaling and prostaglandin production. Substrate-selective COX-2 inhibitors increase brain eCB levels without decreasing prostaglandin levels and cause CB1-dependent anxiolytic actions (Hermanson *et al*, 2014). Whether COX-2-regulated eCBs regulate behavioral stress responses is not yet known, however, COX-2 is an immediate early gene whose expression is upregulated by stress, suggesting that this pathway could be highly relevant during stress exposure. Similar to this approach, a non-canonical pathway of eCB metabolism has recently been identified to potentially be important for the regulation of eCB signaling under conditions of chronic stress. Here the protein-tyrosine phosphatase 1B (PTP1B) was found to regulate eCB production through an mGluR5-dependent pathway, which was increased following chronic stress, and inhibition of PTP1B was found to partially rescue the stress-induced reductions in amygdalar eCB levels and reverse stress-induced anxiety (Qin *et al*, 2015). As such, the possibility of therapeutic interventions of the eCB system in the context of stress-related psychiatric conditions opens many doors to novel therapeutics.

In summary, the eCB system is clearly an important signaling system integrated into the neural regulation of the stress response. This is consistent with the fact that humans have been consuming cannabis for centuries, largely because of its stress-reducing qualities (Green *et al*, 2003). As it is becoming increasingly appreciated that eCB signaling likely represents a mediator of homeostasis, both at the synaptic level and at the systems level of emotional regulation, it will be important moving forward to understand whether perturbations in this system could be important for a wide array of stimuli and disturbances that can lead to changes in stress sensitivity and anxiety, such as obesity and other chronic diseases. A combined approach of pharmacology, genetics, and advanced neuroscientific techniques will hopefully pave the way for our growing understanding of the importance of eCB signaling to keep the brain calm and help us deal with the rapidly growing burden of stress in our society.

FUNDING AND DISCLOSURE

MNH and JSB receive operating funds from the Canadian Institutes of Health Research and MNH holds a Tier II Canada Research Chair and SP from the National Institutes of Health (MH090412 and R21 MH103515). MM receives salary support from an Eyes High Fellowship from the University of Calgary. MNH consults with Pfizer; all the other authors declare no financial conflicts. The funding sources had no influence on the writing of this manuscript.

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