Published in final edited form as:

Neuroscience. 2012 March 1; 204: 5–16. doi:10.1016/j.neuroscience.2011.12.030.

Endocannabinoid Signaling, Glucocorticoid-Mediated Negative Feedback and Regulation of the HPA Axis

M. N. Hill^{1,2,3,*} and J. G. Tasker⁴

¹Department of Cell Biology & Anatomy, University of Calgary, Calgary, Alberta, Canada T2N4N1

²Department of Psychiatry, University of Calgary, Calgary, Alberta, Canada T2N4N1

³Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada T2N4N1

⁴Neuroscience Program and Department of Cell and Molecular Biology, Tulane University, New Orleans, Louisiana, USA 70118

Abstract

The hypothalamic-pituitary-adrenal (HPA) axis regulates the outflow of glucocorticoid hormones under basal conditions and in response to stress. Within the last decade, a large body of evidence has mounted indicating that the endocannabinoid system is involved in the central regulation of the stress response; however, the specific role endocannabinoid signalling plays in phases of HPA axis regulation, or the neural sites of action mediating this regulation, was not mapped out until recently. This review aims to collapse the current state of knowledge regarding the role of the endocannabinoid system in the regulation of the HPA axis to put together a working model of how and where endocannabinoids act within the brain to regulate outflow of the HPA axis. Specifically, we discuss the role of the endocannabinoid system in the regulation of the HPA axis under basal conditions, activation in response to acute stress and glucocorticoid-mediated negative feedback. Interestingly, there appears to be some anatomical specificity to the role of the endocannabinoid system in each phase of HPA axis regulation, as well as distinct roles of both anandamide and 2-arachidonoylglycerol in these phases. Ultimately, the current level of information indicates that endocannabinoid signalling acts to suppress HPA axis activity through concerted actions within the prefrontal cortex, amygdala and hypothalamus.

Stress, the HPA Axis and Glucocorticoid Feedback

The stress response to disruption of physiological homeostasis or to a perceived threat to homeostasis involves a coordinated activation of a constellation of physiological systems designed to increase the survival of the organism. The common stress response to both physiological and psychological stressors can be summarized generally as the concerted activation of a two-pronged physiological defense mechanism, 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. The neuroendocrine stress response is mediated by activation

© 2012 IBRO. Published by Elsevier Ltd. All rights reserved.

*Direct correspondence to: Matthew Hill, Ph.D., Assistant Professor, Departments of Cell Biology & Anatomy and Psychiatry, The Hotchkiss Brain Institute, University of Calgary, 3330 Hospital Drive NW, Calgary AB, Canada T2N4N1, mnhill@ucalgary.ca; Tel.: 403-220-8466.

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

of the hypothalamic-pituitary-adrenal (HPA) axis, which results in an increase in circulating corticosteroids and corticosteroid coordination of activity in multiple target organ systems (Pecoraro et al., 2006).

In the HPA response to stress, stressful stimuli cause the activation of neural inputs to corticotropin releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus (PVN), which leads to CRH and, under some conditions, vasopressin neurosecretion from axon terminals in the basal hypothalamus into the pituitary portal circulation. Portal blood-borne CRH and vasopressin act to stimulate cells of the anterior pituitary that produce adrenocorticotrophic hormone (ACTH), which causes ACTH release into the systemic circulation. Circulating ACTH then stimulates the synthesis of corticosteroids in the cortex of the adrenal glands, which causes an increase in corticosteroid secretion into the blood. Systemic corticosteroids then elicit both rapid and protracted actions in target tissues throughout the organism, including in the brain. Both long-lasting corticosteroid effects, induced by activation of the “classical” intracellular corticosteroid receptors and transcriptional steroid actions, and rapid corticosteroid actions, mediated by putative membrane-associated glucocorticoid receptors and non-genomic steroid actions, have been described in the brain (Tasker and Herman, 2011, Pecoraro et al., 2006).

The HPA axis is under negative feedback control by circulating glucocorticoids. Glucocorticoid feedback regulation of the HPA axis can occur directly at the level of the hypothalamus (Evanson et al., 2010, Jones et al., 1977, Keller-Wood and Dallman, 1984) and pituitary (Russell et al., 2010, Cole et al., 2000), as well as at upstream limbic structures such as the hippocampus (Sapolsky et al., 1984, Jacobson and Sapolsky, 1991, Furay et al., 2008), paraventricular thalamus (PVT; Jaferi et al., 2003; Jaferi and Bhatnagar, 2006) and prefrontal cortex (Hill et al., 2011, Radley and Sawchenko, 2011). Outputs from the PFC and hippocampus/subiculum comprise excitatory projections from principal neurons that transit to the PVN, and reverse their sign, via inhibitory relays in the bed nucleus of the stria terminalis (BNST) and peri-PVN hypothalamic regions (Radley and Sawchenko, 2011, Ulrich-Lai and Herman, 2009). Glucocorticoid actions in these structures, therefore, should be excitatory in order to exert an inhibitory influence on PVN CRH neurons and the HPA axis. In contrast, the direct negative feedback actions of glucocorticoids in the PVN and pituitary are inhibitory (Evanson et al., 2010, Di et al., 2003). Interestingly, the involvement of higher limbic structures in the negative glucocorticoid feedback control of the HPA axis appears to be specific to the HPA response to psychological stressors, and not physiological stressors, based on lesion studies and studies in mice with conditional forebrain cortical and limbic knockout of the glucocorticoid receptor (Diorio et al., 1993; Furay et al., 2008). Rapid glucocorticoid regulation of hypothalamic CRH neurons (Di et al., 2003) as well as hippocampal pyramidal neurons (Hu et al., 2010, Karst et al., 2005), prefrontal cortical pyramidal neurons (Hill et al., 2011, Yuen et al., 2009), and principal neurons of the basolateral amygdala (Karst et al., 2010) is mediated largely by modulation of excitatory and/or inhibitory synaptic inputs to these neurons. In several cases, rapid glucocorticoid actions have been demonstrated to include stimulation of endocannabinoid synthesis and engagement of the endocannabinoid system in the modulation of synaptic inputs (Hill et al. 2010a, Karst et al., 2010, Di et al., 2003); for review, see Tasker and Herman, 2011).

Endocannabinoid Signaling

The endocannabinoid system is a lipid signaling system throughout the brain and body that was first discovered as the biochemical target of delta⁹-tetrahydrocannabinol (THC), the psychoactive constituent of cannabis (Pertwee, 2008). The endocannabinoid system is composed of two G-protein coupled receptors, denoted CB₁ and CB₂ receptors, which exhibit distinct patterns of distribution (Matsuda et al., 1990, Munro et al., 1993). The CB₁

receptors are widely expressed throughout the brain, but is also found in many cell types and organ systems of the periphery as well, including all major endocrine glands (Herkenham et al., 1991, Belloccchio et al., 2008). The CB₂ receptors are traditionally viewed as exhibiting peripheral expression patterns exclusively, particularly on organs involved in the immune response such as leukocytes and the spleen (Atwood and Mackie, 2010, Patel et al., 2010); however, recent evidence has questioned this view and there is emerging evidence for the expression of CB₂ receptors in the brain (Onaivi et al., 2011, Xi et al., 2011, Van Sickle et al., 2005). Given that all of the research examining the endocannabinoid system and the HPA axis has focused on the CB₁ receptor, this review will focus exclusively on the CB₁ receptor.

Within the brain, the CB₁ receptor is predominately expressed on axon terminals of a variety of neuronal populations, including glutamatergic, GABAergic and monoaminergic neurons (Freund et al., 2003). Activation of the CB₁ receptor results in a suppression of adenylate cyclase activity and calcium influx into the axon terminal; thus, CB₁ receptor signaling functions to suppress neurotransmitter release into the synapse (Freund et al., 2003). In addition to the ability of THC to activate CB₁ receptors, there are at least two endogenous ligands that activate the CB₁ receptor (termed endocannabinoids). The two ligands that are widely accepted as endocannabinoids, *N*-arachidonyl ethanolamine (anandamide; AEA; Devane et al., 1992) and 2-arachidonoylglycerol (2-AG; Sugiura et al., 1995), are arachidonate-derived signaling lipids, which in the brain are synthesized in the post-synaptic membrane and released in a retrograde fashion to activate presynaptically located CB₁ receptors (Alger, 2002, Wilson and Nicoll, 2002). The synthesis of AEA and 2-AG is believed to be driven by the cleavage of membrane-associated phospholipid head groups by activation of specific enzymes. The activation of these biosynthetic enzymes can be driven by activity-dependent depolarization and increased intracellular calcium signaling or through metabotropic receptor activation (Freund et al., 2003). The biosynthesis of 2-AG is mediated by the generation of diacylglycerol, via the actions of either phospholipase C (PLC) or phospholipase D (PLD), which is subsequently converted to 2-AG via the actions of DAG lipase (Hillard, 2000, Sugiura et al., 2002, Di Marzo, 2008). The pathways mediating AEA synthesis are less well understood. To date, three distinct and independent mechanisms have been found to generate AEA (Okamoto et al., 2004, Liu et al., 2006, Simon and Cravatt, 2006); however, the pathway that is primarily responsible for neuronal AEA synthesis is not currently known (see Ahn et al., 2008, Bisogno, 2008 for details on putative biosynthetic pathways of AEA). The functional lifespan of endocannabinoids in the synapse is determined by their metabolism by specific enzymatic pathways. Fatty acid amide hydrolase (FAAH) is the primary catabolic enzyme of AEA, and hydrolyzes AEA into ethanolamine and arachidonic acid (Deutsch et al., 2002, Ueda, 2002). 2-AG is primarily metabolized by monoacylglyceride lipase (MAG lipase) to form glycerol and arachidonic acid (Dinh et al., 2002, Ueda, 2002).

While the physiological function of AEA and 2-AG are very similar, based upon differences in binding affinity, pharmacokinetics and signaling efficacy, we and others have proposed that AEA represents a “tonic” signal that gates and regulates transmitter release under steady-state conditions, while 2-AG represents a “phasic” signal that is brought on-line during sustained neuronal depolarization and is involved in many forms of synaptic plasticity (Ahn et al., 2008, Gorzalka et al., 2008). This concept is highlighted by the fact that AEA and 2-AG possess distinct biosynthetic and metabolic pathways, and pharmacological or genetic modulation of their signaling produces distinct behavioural and physiological responses (Ahn et al., 2008, Long et al., 2009a, Long et al., 2009b).

With respect to regulation of the HPA axis, the endocannabinoid system is widely distributed throughout the cortico-limbic and hypothalamic circuitry that regulates activation

of the HPA axis (Hill and McEwen, 2010, Gorzalka et al., 2008). Within this circuitry, endocannabinoid signaling has been found to regulate both excitatory and inhibitory transmitter release, and in general, the predominant effects of endocannabinoid signaling are to constrain activation of the HPA axis (Hill et al., 2010c). However, research has identified site-specific roles, and divergent functions of AEA and 2-AG, with respect to HPA axis regulation in the context of basal function, activation in response to stress and termination during the HPA recovery phase. Each of these phases will be discussed independently.

Endocannabinoid Signaling and Basal Regulation of the HPA Axis

Studies employing mice deficient in CB₁ receptors (CB₁ KO mice) or treated with a CB₁ receptor antagonist generally indicate that a disruption of CB₁ receptor function increases basal drive on the HPA axis. Specifically, acute treatment with a CB₁ receptor antagonist to an otherwise unstressed rodent has been reliably found to increase circulating levels of ACTH and corticosterone in a dose-dependent manner during the *nadir* of the diurnal cycle (Atkinson et al., 2010, Hill et al., 2010b, Manzanares et al., 1999, Gonzalez et al., 2004, Patel et al., 2004, Wade et al., 2006, Steiner et al., 2008, Newsom et al., 2011). These data suggest that there is an endocannabinoid tone, which, under steady-state conditions, constrains activation of the HPA axis and, when disrupted, increases HPA axis outflow. More detailed studies have revealed that this effect is likely due to basal endocannabinoid suppression of the peak amplitudes in the pulsatility of ACTH and corticosterone release and not to a regulation of the frequency of pulses (Atkinson et al., 2010). Furthermore, the ability of CB₁ receptor antagonism to increase HPA axis activity appears to be greater during the daily *nadir*, suggesting that endocannabinoids may contribute more to the decrease in HPA axis outflow during the diurnal trough, than to regulating basal activity during the diurnal peak (Atkinson et al., 2010). Consistent with these findings, CB₁ KO mice have generally been found to exhibit increased basal HPA axis activity. Specifically, CB₁ KO mice have increased CRH mRNA within the PVN (Cota et al., 2003, Cota et al., 2007) and, consistent with the pharmacological studies, several studies have similarly demonstrated that CB₁ KO mice have increased circulating levels of ACTH and corticosterone during the daily *nadir* (Barna et al., 2004, Haller et al., 2004, Steiner et al., 2008), but also during the diurnal peak (Cota et al., 2007). Some studies have reported no difference between wild-type and CB₁ KO mice in basal ACTH and/or corticosterone levels (Fride et al., 2005, Wade et al., 2006, Aso et al., 2008); however, these discrepancies could be due to differences in strain, breeding/outbreeding protocols and ambient laboratory conditions. Regardless, the general consensus in this area is that there is an endocannabinoid tone that acts to constrain HPA axis under basal conditions, particularly during the *nadir* of the diurnal cycle and to some degree during the diurnal peak, and disruption of this tone increases HPA axis activity.

With respect to the site of action of tonic endocannabinoid suppression of HPA axis activity, the current body of evidence suggests that this effect is centrally mediated. While there is evidence that CB₁ receptors are present in the pituitary and adrenal glands, where they may regulate the synthesis and/or release of ACTH and corticosterone (Ziegler et al., 2010, Pagotto et al., 2001, Cota et al., 2007), the HPA stimulating effects of CB₁ receptor antagonism can be replicated by intra-cerebroventricular administration (Manzanares et al., 1999), indicating a central site of action. Consistent with this hypothesis, systemic administration of a CB₁ receptor antagonist increases neuronal activation (as indicated by the induction of the immediate early gene *c-fos*) in the PVN (Patel et al., 2004, Doyon et al., 2006, Newsom et al., 2011), suggesting that the increase in HPA axis activity is mediated by an activation of CRH neurosecretory cells in the PVN, although the site of endocannabinoid action may be in upstream excitatory neural circuits.

Within the PVN, CB₁ receptors are localized to glutamatergic terminals impinging upon CRH neurosecretory neurons, and CB₁ receptor activation decreases glutamate release onto these neurons (Wamsteeker et al., 2010, Di et al., 2003). Theoretically, these excitatory synapses would be an ideal site for endocannabinoid gating of basal HPA axis activity; however, there does not appear to be an endocannabinoid tone within this micro-circuit that contributes to the regulation of basal HPA axis activity, as CB₁ receptor antagonism in PVN slices does not increase excitatory synaptic inputs to CRH neurons (Di et al., 2003) and local administration of a CB₁ receptor antagonist in the PVN does not increase basal HPA axis drive (Evanson et al., 2010). These data suggest, therefore, that tonic regulation of the HPA axis by endocannabinoids occurs at an extrahypothalamic site that communicates with the PVN.

In addition to the PVN, *c-fos* studies have indicated that acute administration of a CB₁ receptor antagonist increases neuronal activation in limbic circuits that feed into the PVN, particularly the amygdala and the prefrontal cortex (Newsom et al., 2011, Alonso et al., 1999, Singh et al., 2004, Patel et al., 2005a). Local administration of a CB₁ receptor antagonist into the medial prefrontal cortex, however, has no effect on basal HPA axis function (Hill et al., 2011), indicating that the medial prefrontal cortex is also not the location by which endocannabinoid signaling tonically regulates basal HPA axis activity. Interestingly, administration of a CB₁ receptor antagonist in the basolateral nucleus of the amygdala (BLA; Ganon-Elazar and Akirav, 2009, Hill et al., 2009a), but not in the medial or central nuclei of the amygdala (Hill et al., 2009a), increases HPA axis activity in non-stressed animals. These data would indicate that an endocannabinoid tone within the BLA tonically gates excitation of this structure, and that disruption of CB₁ receptor function in the BLA increases its intrinsic excitability, which ultimately results in the activation of the PVN. While the BLA does not anatomically project directly to the PVN, it does regulate PVN activity through a series of trans-synaptic pathways including the central and medial nuclei of the amygdala, the bed nucleus of the stria terminalis and neighbouring hypothalamic nuclei (Herman et al., 2005, Ulrich-Lai and Herman, 2009), and activation of the BLA is sufficient to increase HPA axis activity (Feldman et al., 1982). Consistent with this, CB₁ receptors in the BLA are located on both glutamatergic and GABAergic terminals (Marsicano and Lutz, 1999, Katona et al., 2001, McDonald and Mascagni, 2001, Azad et al., 2003, Domenici et al., 2006), but electrophysiological work has demonstrated that the ability of CB₁ receptor signaling to suppress glutamate signaling in this nucleus overrides the suppression of GABA signaling, as the net effect of CB₁ receptor activation in the BLA is a reduction in the firing activity of principal neurons (Azad et al., 2003). As such, our current working model is that, under steady-state conditions, there is an endocannabinoid tone that gates excitatory inputs to principal neurons in the BLA and thus constrains the excitability of the BLA under non-stressed conditions. Disruption of this endocannabinoid tone increases excitation in the BLA, and the increased outflow of projection neurons from the BLA stimulates the activation of the PVN and results in an increase in HPA activity. As AEA is believed to represent the “tonic” signaling molecule of the endocannabinoid system, our current hypothesis is that AEA signaling within the BLA is a distal “gatekeeper” of basal HPA axis activity.

Endocannabinoid Signaling and Stress-induced Activation of the HPA Axis

Consistent with the hypothesis that AEA signaling within the BLA represents a “gatekeeper” over HPA axis activity, evidence has also mounted that a rapid loss of this AEA signal in the BLA is involved in the natural activation of the HPA axis in response to stress. Specifically, exposure to stress results in a reduction in the tissue content of AEA in the amygdala (Patel et al., 2005b, Rademacher et al., 2008, Hill et al., 2009a), possibly through a rapid induction of FAAH-mediated AEA hydrolysis (Hill et al., 2009a). The

magnitude of the decline in AEA content within the amygdala negatively correlates with the extent of HPA axis activation, such that larger reductions in amygdala AEA levels in response to stress are related to greater increases in corticosterone secretion (Hill et al., 2009a). Furthermore, local administration of a FAAH inhibitor in the BLA, but not in the central or medial nuclei of the amygdala, attenuates stress-induced activation of the HPA axis, indicating that AEA hydrolysis in the BLA in response to stress contributes to activation of the HPA axis (Hill et al., 2009a). These data are consistent with the aforementioned hypothesis, and suggest that this “gatekeeper” role of AEA signaling in the BLA is modified by exposure to stress to facilitate the neuroendocrine response to stress. Additionally, local administration of a CB₁ receptor agonist directly into the BLA similarly suppresses stress-induced activation of the HPA axis (Ganon-Elazar and Akirav, 2009; Hill et al., 2009a), supporting the hypothesis that CB₁ receptor signaling in the BLA counters HPA axis activity. Taken together, these data demonstrate that AEA signaling in the BLA plays an important role in the regulation of the HPA axis (see Figure 1). Under steady-state conditions, there is an AEA tone within the BLA that gates incoming excitatory neurotransmission. Disruption of this AEA tone following exposure to stress, either through the blockade of CB₁ receptor signaling or a reduction in AEA content, increases the activation of principal neurons in the BLA and results in an increase in HPA axis activation and in the release of glucocorticoid hormones into the circulation.

Endocannabinoid Signaling and Glucocorticoid-mediated Negative Feedback

Once glucocorticoids are released into the circulation, they activate multiple negative feedback loops both within central and peripheral components of the HPA axis and within extrahypothalamic regions in the brain. This feedback process occurs within distinct temporal windows (Keller-Wood and Dallman, 1984; Pecoraro et al., 2006). Rapid negative feedback processes occur within a few minutes (typically under 10 min), are not sensitive to protein synthesis inhibition and appear to be mediated by non-classical glucocorticoid actions at the membrane level (Keller-Wood and Dallman, 1984; Dallman, 2005). Delayed negative feedback occurs over a longer duration (minutes to hours), is driven by changes in gene expression and is mediated by classical actions of glucocorticoids within the nucleus (Keller-Wood and Dallman, 1984; Pecoraro et al., 2006). At the adrenal and pituitary levels, there is negative feedback initiated by glucocorticoids that acts to suppress the release of both ACTH from the pituitary and corticosteroids from the adrenal cortex to limit the release of HPA hormones. Within the brain, there is a complex process of glucocorticoid feedback that involves both rapid and delayed components at multiple sites (Tasker and Herman, 2011; Pecoraro et al., 2006). As discussed previously, within the PVN of the hypothalamus there is both a rapid and delayed feedback process that involves a suppression of the excitatory drive to CRH neurosecretory cells and a down-regulation of CRH and vasopressin mRNA transcription in these same neurons, respectively. The down-regulation of CRH and vasopressin mRNA transcription is directly mediated by genomic actions of glucocorticoids and occurs over a period of a couple of hours. The rapid feedback component, however, is driven by non-genomic actions of glucocorticoids interacting with membrane-associated glucocorticoid receptors located on the extracellular surface of CRH neurosecretory cells within the PVN. Activation of these membrane glucocorticoid receptors results in the generation of a retrograde signal that traverses back across the synaptic cleft to axon terminals of excitatory afferents and suppresses the release of glutamate onto CRH neurons, suppressing the excitation of these cells and the release of CRH. Multiple lines of evidence have demonstrated that this fast feedback inhibition of glucocorticoids locally within the PVN is mediated by an endocannabinoid signal (for detailed discussion of this phenomenon please refer to Tasker and Herman, 2011).

First, bath application of glucocorticoids to PVN slices results in a rapid suppression of glutamate-mediated excitatory synaptic currents in CRH neurons, which is completely abrogated by co-application of a CB₁ receptor antagonist (Wamsteeker et al., 2010, Di et al., 2003, Malcher-Lopes et al., 2006). This indicates that glucocorticoids act to mobilize endocannabinoids within the PVN to dampen excitatory synaptic input to CRH neurons. Consistent with this hypothesis, glucocorticoids have been found to increase the content of both AEA and 2-AG in PVN slices *in vitro* (Malcher-Lopes et al., 2006) and in whole hypothalamic sections *in vivo* (Hill et al., 2010a). Furthermore, 30-min exposure to restraint stress similarly increases 2-AG content within the hypothalamus *in vivo* (Evanson et al., 2010). At the systems level, while local administration of a CB₁ receptor antagonist does not affect basal drive on the HPA axis, it is capable of completely preventing the ability of intra-PVN glucocorticoid administration to dampen stress-induced activation of the HPA axis (Evanson et al., 2010). Furthermore, CB₁ receptor KO mice exhibit a larger peak ACTH and corticosterone response following acute stress, suggesting that a loss of CB₁ receptors reduces the fast feedback inhibition and increases the magnitude and duration of the HPA axis response to acute stress (Hill et al., 2011, Barna et al., 2004, Haller et al., 2004, Uriguen et al., 2004, Aso et al., 2008, Steiner et al., 2008). As such, these data create a compelling argument that endocannabinoids mediate fast-feedback inhibition of the HPA axis by glucocorticoids locally within the PVN through a non-genomic mechanism by which glucocorticoids induce endocannabinoid mobilization to suppress excitatory input to CRH neurons.

Outside of the PVN, there is also evidence that endocannabinoids are involved in glucocorticoid-feedback inhibition of the HPA axis. Within the amygdala for example, as it has been established that AEA signaling in the BLA is an important component of basal HPA axis activity and its activation in response to stress, it is interesting to note that glucocorticoids and stress exposure exert oppositional effects on AEA content. Specifically, as mentioned previously, acute exposure to stress reduces AEA content in the basolateral nucleus of the amygdala (Patel et al., 2005b, Rademacher et al., 2008, Hill et al., 2009a), however administration of glucocorticoids in the absence of stress actually causes a rapid increase in AEA content within the amygdala (Hill et al., 2010a). One interpretation of these data is that stress, through a non-glucocorticoid pathway (possibly CRH or norepinephrine), rapidly suppresses AEA signaling within the BLA to facilitate activation of the HPA axis, and once glucocorticoid levels begin to rise they act to increase AEA content in the amygdala and thus normalize the reduction produced by stress exposure. While this process remains to be determined experimentally, a recent report showed that exposure of BLA slices from stressed animals to corticosterone *in vitro* results in a suppression of afferent glutamatergic input through an endocannabinoid mechanism (Karst et al., 2010). As such, the ability of glucocorticoids to rapidly increase AEA content within the BLA may be one of the mechanisms by which glucocorticoids reduce neuronal activity within the amygdala (Henckens et al., 2010) and contribute to termination of the stress response.

In addition to these rapid, non-genomic effects of glucocorticoids to suppress activation of the HPA axis, the medial prefrontal cortex and hippocampus also represent two neuroanatomical sites integral for delayed feedback inhibition of the HPA axis. Glucocorticoid receptors are abundantly expressed in both the prefrontal cortex and hippocampus, and activation of these receptors inhibits activation of the HPA axis, while lesioning of either of these structures impairs normative recovery of the HPA axis following exposure to stress (Radley and Sawchenko, 2011, Diorio et al., 1993, Herman and Mueller, 2006, Radley et al., 2006). An elegant series of anatomical studies has demonstrated that both of these structures suppress HPA axis activity through activation of glutamatergic projection neurons to inhibitory relays to the PVN within the bed nucleus of the stria terminalis (BNST; Radley and Sawchenko, 2011). As such, glucocorticoids facilitate

neuronal activity within the medial prefrontal cortex (Hill et al., 2011, Yuen et al., 2009) and hippocampus (Karst et al., 2005) to increase the outflow of projection neurons in these structures to suppress HPA axis activity and terminate the stress response.

In the prefrontal cortex, a clear role of endocannabinoid signaling in the glucocorticoid-mediated negative feedback inhibition of the HPA axis has been demonstrated. Specifically, exposure to stress was found to increase 2-AG content, but not AEA, within the prefrontal cortex in a glucocorticoid-dependent manner (Hill et al., 2011). Unlike what was seen in the PVN (Di et al., 2003), however, this ability of glucocorticoids to increase endocannabinoid content was not rapid and involved genomic actions of glucocorticoids, as it was blocked by the classical intracellular glucocorticoid receptor antagonist RU-486 (Hill et al., 2011). Whereas local administration of a CB₁ receptor antagonist did not change the peak corticosterone responses to acute stress, it did prolong the duration of elevated corticosterone secretion following termination of the stress, indicating that the recruitment of 2-AG by glucocorticoids in the mPFC contributes to termination of the stress response (Hill et al., 2011). The ability of endocannabinoids in the prefrontal cortex to contribute to the termination of the stress response appears to be due to modulation of local excitability, as CB₁ receptors were found on GABAergic terminals clustered around pyramidal neurons in the prefrontal cortex, and bath application of corticosterone to prefrontal cortical slices resulted in a CB₁ receptor-dependent reduction of inhibitory tone in these cells (Hill et al., 2011). Again, unlike in the hypothalamus, this effect appeared to be mediated by genomic actions of glucocorticoids, as it was seen following 1 h of glucocorticoid application (Hill et al., 2011). While the exact nature of how glucocorticoids may interact with the endocannabinoid system at a genomic level has yet to be determined, preliminary evidence indicates that glucocorticoids can downregulate MAG lipase activity (Shrestha and Hillard, 2010), so it is possible that glucocorticoids may act to suppress 2-AG metabolism and thus increase the synaptic availability of 2-AG. As such, these data demonstrate that endocannabinoid system is also recruited in the prefrontal cortex by glucocorticoids following exposure to stress, and that the endocannabinoid-induced increase in endocannabinoid signaling increases the activity of prefrontal cortical outputs and contributes to termination of the stress response and glucocorticoid-mediated negative feedback.

In the hippocampus, it is possible that a similar phenomenon is occurring, however this remains to be fully determined. Currently, it has been shown that exposure to acute stress increases 2-AG content within the hippocampus through activation of genomic glucocorticoid receptors (Wang et al., 2011). Furthermore, both stress and corticosterone exposure (*in vivo* and *in vitro*) increase endocannabinoid-mediated suppression of GABAergic transmission within the hippocampus (Wang et al., 2011). However, it has yet to be determined if this increase in endocannabinoid signaling can influence the excitability of projection neurons from the hippocampus to the BNST that are involved in the termination of the stress response (Radley and Sawchenko, 2011), or if local antagonism of CB₁ receptors in the hippocampus can modulate stress-induced HPA axis activity or glucocorticoid-mediated negative feedback. Future research should examine if a similar endocannabinoid-mechanism of glucocorticoid feedback inhibition is occurring in the hippocampus as what has been determined in the prefrontal cortex (Hill et al., 2011).

Taken together, these data demonstrate that endocannabinoids play an important role in glucocorticoid-mediated negative feedback (see Figure 2). In addition to the data detailed above, it has also been reported that CB₁ receptor knockout mice also exhibit deficits in dexamethasone-mediated suppression of HPA axis activity (Cota et al., 2007), further demonstrating an important role of the endocannabinoid system in glucocorticoid feedback inhibition of the HPA axis. Interestingly, the current data indicate that the endocannabinoid

system appears to contribute to both the short and long feedback loops of glucocorticoid feedback inhibition, and does so through distinct mechanisms. Within the PVN, and possibly in the amygdala, a rapid induction of endocannabinoid activity through a non-genomic glucocorticoid mechanism contributes to the fast feedback inhibition of the HPA axis, while in the prefrontal cortex, and possibly in the hippocampus, a delayed increase in endocannabinoid activity, through a genomic glucocorticoid mechanism, contributes to the long-loop glucocorticoid feedback inhibition pathway. As such, the endocannabinoid system appears to represent one of the synaptic workhorses of glucocorticoids, bridging postsynaptic effects of glucocorticoids to presynaptic regulation of excitability within a given circuit (Hill and McEwen, 2009).

Model of Endocannabinoid Actions in HPA Axis Regulation and Glucocorticoid-Mediated Negative Feedback

The body of research detailed herein demonstrates a critical and complex role of the endocannabinoid system in the regulation of HPA axis activity and glucocorticoid-mediated negative feedback. When looking at the complete picture of the role of endocannabinoid signaling in HPA axis regulation, a ying-yang role of AEA and 2-AG emerges and suggests a fluid model of how this system integrates into the HPA axis (Figure 3). Under steady-state conditions, there is an AEA tone in the BLA that gates excitation of BLA principal neurons and suppresses BLA activity in the absence of stress. Following exposure to stress, AEA content within the BLA rapidly decreases, which disinhibits the BLA and results in an activation of the HPA axis and glucocorticoid hormone secretion into the circulation. As glucocorticoid hormone concentrations rise and penetrate the brain, they bind to membrane-associated receptors in the PVN, and possibly in the amygdala, to rapidly induce endocannabinoid synthesis. Within the PVN, this increase in endocannabinoid signaling suppresses excitatory synaptic drive to the CRH neurons and acts to mediate the fast feedback inhibition of the HPA axis. Within the amygdala, this increase in endocannabinoid signaling functions to decrease the excitatory drive to principal neurons of the BLA and decrease the facilitatory influence this nuclei has on the HPA axis. In the prefrontal cortex, and possibly in the hippocampus, glucocorticoids produce a delayed increase in 2-AG content by activating genomic receptors, possibly through a down-regulation of MAG lipase activity. This delayed increase in 2-AG acts to increase the excitability of projection neurons by suppressing local inhibitory tone on these neurons. This increase in outflow of the prefrontal cortex and hippocampus activates inhibitory relays in the BNST that suppress neuronal activation in the PVN. Collectively, this multi-site process results in the termination of the HPA axis and limits the duration and magnitude of glucocorticoid secretion in response to stress. In addition, this model also reveals a divergent pattern of endocannabinoid regulation which indicates putatively distinct role of AEA and 2-AG. AEA signalling exerts a tonic suppression over the HPA axis, while 2-AG is recruited by glucocorticoids to turn off the HPA axis following cessation of stress. As such, this hypothetical model reveals multiple sites of action by which a disruption in endocannabinoid signaling could result in an increase in HPA axis activity and illustrates how endocannabinoid activity can regulate the HPA axis at multiple levels and in distinct phases of the stress response.

Conclusions and Future Directions

From this overview, we are able to determine multiple areas of research that are required to fully understand the interaction between endocannabinoids and the HPA axis. First, it remains to be determined what the mechanism is through which stress reduces AEA content. This phenomenon does not appear to be mediated by glucocorticoids, and happens upstream of HPA axis activation (Hill et al., 2009a), thus likely involves local signaling systems that

are activated by stress stimulation prior to the activation of the HPA axis, such as CRH or norepinephrine release within the amygdala. Second, in addition to the identified structures, it is important to determine if endocannabinoid signaling operates at additional sites to regulate HPA axis function. As mentioned, there is no evidence to date that shows that manipulation of endocannabinoid signaling in the hippocampus modulates HPA axis function. One study has demonstrated that CB₁ receptor knockout mice exhibit a downregulation of glucocorticoid receptors within the hippocampus (a condition that is associated with impaired feedback inhibition of the HPA axis; Sapolsky et al., 1984, Liu et al., 1997, Mizoguchi et al., 2003); however, whether this downregulation is secondary to glucocorticoid hypersecretion or a contributing factor to HPA axis hyperactivity in CB₁ receptor knockout mice is not known. In addition to the hippocampus, there is no evidence regarding the role of endocannabinoid signaling in many structures known to be integral for HPA axis regulation, such as in the BNST (Radley and Sawchenko, 2011, Choi et al., 2007), in midline thalamic nuclei such as the paraventricular nucleus of the thalamus (Bhatnagar et al., 2000, Jaferi and Bhatnagar, 2006, Jaferi et al., 2003), in the lateral septum (Singewald et al., 2011) and in hypothalamic nuclei such as the medial preoptic area (Viau and Meaney, 1996) and dorsomedial hypothalamic nucleus (Keim and Shekhar, 1996, Bailey and Dimicco, 2001). Third, there is little information regarding the role of endocannabinoid signaling in different forms of stress induction. To date, all of the studies examining the role of the endocannabinoid system in HPA axis regulation have focused on psychogenic stressors and not physiological stressors. One recent study reported that inhibition of FAAH did not modulate HPA axis activation in response to an immunological challenge (Kerr et al., 2011), but there is no information regarding the role of endocannabinoids in HPA activation by other forms of physiological stress such as osmotic dysregulation or pain. Finally, neuroanatomical studies are required to adequately understand where the molecular components of the endocannabinoid system exist within known circuits regulating the HPA axis. In the PVN, CB₁ receptors are known to exist on glutamatergic inputs to CRH neurons (Di et al., 2003) and in the prefrontal cortex, CB₁ receptors are clustered on GABAergic terminals impinging on layer V neurons of the prelimbic region, the output neurons that project to subcortical structures such as the BNST (Hill et al., 2011); however, more work is required to understand where CB₁ receptors are located in stress-sensitive neurons that have been widely mapped using *c-fos* induction to comprise the circuitry that regulates HPA axis function (Radley and Sawchenko, 2011, Cullinan et al., 1995, Herman et al., 2005, Spencer et al., 2005).

Finally, there is emerging evidence that stress also recruits the endocannabinoid system in humans, implying a translational validity to the animal studies. Exposure of individuals to a fifteen minute social stressor (the Trier Social Stress Test) resulted in an increase in the circulating levels of 2-AG (Hill et al., 2009b) that was similar to the increase in central 2-AG content seen in rodents following exposure to stress (Evanson et al., 2010, Hill et al., 2011, Wang et al., 2011). These data suggest that endocannabinoid signaling is engaged by stress in humans; however, it remains to be determined whether this increase in 2-AG content in the blood reflects central changes in endocannabinoid signaling, and whether this increase in 2-AG content is involved in glucocorticoid-mediated feedback inhibition of the HPA axis. A separate study has also found that individuals who carry the C385A polymorphism of the FAAH gene, which increases the proteolytic degradation of FAAH and elevates AEA content (Sipe et al., 2010, Sipe et al., 2002), exhibit a reduction in activation of the amygdala in response to threatening stimuli (Hariri et al., 2009). This finding is consistent with the idea that there is an AEA inhibitory tone within the amygdala that regulates its excitability, and that loss of this tone in response to stress facilitates activation of the amygdala. These data in humans complement and corroborate the preclinical studies demonstrating that local inhibition of FAAH within the amygdala can attenuate the stress response (Hill et al., 2009a). Finally, a recent report has found that administration of a CB₁

receptor antagonist in humans may increase cortisol levels when delivered at high doses (Goodwin et al., 2011), suggesting that endocannabinoid signaling may constrain basal activation of the HPA axis in humans, similar to what has been found in rodents. Taken together, these data provide some initial evidence that endocannabinoid signaling may operate to regulate the HPA axis in a similar fashion in humans as what has been described in rodents (for further review, see the article in this issue by Hillard et al., 2011). This conserved ability of endocannabinoid signaling to regulate HPA axis activity may represent one of the mechanisms by which inhibition of CB₁ receptor signaling in humans can contribute to the incidence of depression in humans (de Mattos Viana et al., 2009, Hill and Gorzalka, 2009), a disease that is often associated with excessive activation or perturbed circadian rhythms of the HPA axis (Stetler and Miller, 2011, Holsboer, 2000).

In addition to the information detailed herein regarding the role of the endocannabinoid system in the regulation of basal HPA activity, stress-evoked activation of the HPA axis, and termination of the HPA response under conditions of acute stress, there is evidence that the endocannabinoid system also contributes to multiple forms of HPA axis regulation under conditions of chronic stress, such as reduced negative feedback inhibition of the PVN parvocellular neurons (Wamsteeker et al., 2010), habituation of stress-induced HPA axis activity (Hill et al., 2010b, Patel et al., 2005b) and increased basal excitatory drive of the HPA axis (Hill et al., 2010b). Similar to the data described for acute stress, these processes also seem to involve endocannabinoid signaling in the amygdala, hypothalamus and prefrontal cortex (Hill et al., 2010b, Wamsteeker et al., 2010, Patel et al., 2004, Patel et al., 2005b). Therefore, the role of the endocannabinoid system in HPA axis regulation extends beyond the acute stress phase to chronic stress plasticity, and the endocannabinoid system plays an integral part in the changing expression of the HPA response to a continually fluctuating and often challenging environment. It remains to be determined whether plasticity of the endocannabinoid system plays an adaptive or maladaptive role in the chronic stress induction of stress-related pathologies.

Acknowledgments

The authors would like to thank Caitlin Riebe for her excellent technical assistance in the designing and rendering of the figures in this manuscript. JGT is the recipient of a National Institute of Mental Health Grant 2R01 MH066958. Additionally, funding support was provided by the Catherine and Hunter Pierson Chair in Neuroscience, Tulane University Research Enhancement Fund to JGT.

References

- Ahn K, McKinney MK, Cravatt BF. Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. *Chem Rev.* 2008; 108:1687–1707. [PubMed: 18429637]
- Alger BE. Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Prog Neurobiol.* 2002; 68:247–286. [PubMed: 12498988]
- Alonso R, Voutsinos B, Fournier M, Labie C, Steinberg R, Souilhac J, Le Fur G, Soubrie P. Blockade of cannabinoid receptors by SR141716 selectively increases Fos expression in rat mesocorticolimbic areas via reduced dopamine D2 function. *Neuroscience.* 1999; 91:607–620. [PubMed: 10366018]
- Aso E, Ozaita A, Valdizan EM, Ledent C, Pazos A, Maldonado R, Valverde O. BDNF impairment in the hippocampus is related to enhanced despair behavior in CB1 knockout mice. *J Neurochem.* 2008; 105:565–572. [PubMed: 18047561]
- Atkinson HC, Leggett JD, Wood SA, Castrique ES, Kershaw YM, Lightman SL. Regulation of the hypothalamic-pituitary-adrenal axis circadian rhythm by endocannabinoids is sexually diergic. *Endocrinology.* 2010; 151:3720–3727. [PubMed: 20534730]
- Atwood BK, Mackie K. CB2: a cannabinoid receptor with an identity crisis. *Br J Pharmacol.* 2010; 160:467–479. [PubMed: 20590558]

- Azad SC, Eder M, Marsicano G, Lutz B, Zieglgansberger W, Rammes G. Activation of the cannabinoid receptor type 1 decreases glutamatergic and GABAergic synaptic transmission in the lateral amygdala of the mouse. *Learn Mem.* 2003; 10:116–128. [PubMed: 12663750]
- Bailey TW, Dimicco JA. Chemical stimulation of the dorsomedial hypothalamus elevates plasma ACTH in conscious rats. *Am J Physiol Regul Integr Comp Physiol.* 2001; 280:R8–15. [PubMed: 11124128]
- Barna I, Zelena D, Arszovszki AC, Ledent C. The role of endogenous cannabinoids in the hypothalamo-pituitary-adrenal axis regulation: in vivo and in vitro studies in CB1 receptor knockout mice. *Life Sci.* 2004; 75:2959–2970. [PubMed: 15454346]
- Bellochio L, Cervino C, Pasquali R, Pagotto U. The endocannabinoid system and energy metabolism. *J Neuroendocrinol.* 2008; 20:850–857. [PubMed: 18601709]
- Bhatnagar S, Viau V, Chu A, Soriano L, Meijer OC, Dallman MF. A cholecystokinin-mediated pathway to the paraventricular thalamus is recruited in chronically stressed rats and regulates hypothalamic-pituitary-adrenal function. *J Neurosci.* 2000; 20:5564–5573. [PubMed: 10884340]
- Bisogno T. Endogenous cannabinoids: structure and metabolism. *J Neuroendocrinol.* 2008; 20(Suppl 1):1–9. [PubMed: 18426492]
- Choi DC, Furay AR, Evanson NK, Ostrander MM, Ulrich-Lai YM, Herman JP. Bed nucleus of the stria terminalis subregions differentially regulate hypothalamic-pituitary-adrenal axis activity: implications for the integration of limbic inputs. *J Neurosci.* 2007; 27:2025–2034. [PubMed: 17314298]
- Cole MA, Kim PJ, Kalman BA, Spencer RL. Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. *Psychoneuroendocrinology.* 2000; 25:151–167. [PubMed: 10674279]
- Cota D, Marsicano G, Tschöp M, Grubler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thone-Reineke C, Ortmann S, Tomassoni F, Cervino C, Nisoli E, Linthorst AC, Pasquali R, Lutz B, Stalla GK, Pagotto U. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest.* 2003; 112:423–431. [PubMed: 12897210]
- Cota D, Steiner MA, Marsicano G, Cervino C, Herman JP, Grubler Y, Stalla J, Pasquali R, Lutz B, Stalla GK, Pagotto U. Requirement of cannabinoid receptor type 1 for the basal modulation of hypothalamic-pituitary-adrenal axis function. *Endocrinology.* 2007; 148:1574–1581. [PubMed: 17194743]
- Cullinan WE, Herman JP, Battaglia DF, Akil H, Watson SJ. Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience.* 1995; 64:477–505. [PubMed: 7700534]
- Dallman MF. Fast glucocorticoid actions on the brain: Back to the future. *Front Neuroendocrinol.* 2005; 26:103–108. [PubMed: 16242180]
- de Mattos Viana B, Prais HA, Daker MV. Melancholic features related to rimonabant. *Gen Hosp Psychiatry.* 2009; 31:583–585. [PubMed: 19892218]
- Deutsch DG, Ueda N, Yamamoto S. The fatty acid amide hydrolase (FAAH). *Prostaglandins Leukot Essent Fatty Acids.* 2002; 66:201–210. [PubMed: 12052036]
- Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science.* 1992; 258:1946–1949. [PubMed: 1470919]
- Di Marzo V. Endocannabinoids: synthesis and degradation. *Rev Physiol Biochem Pharmacol.* 2008; 160:1–24. [PubMed: 18481028]
- Di S, Malcher-Lopes R, Halmos KC, Tasker JG. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. *J Neurosci.* 2003; 23:4850–4857. [PubMed: 12832507]
- Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A.* 2002; 99:10819–10824. [PubMed: 12136125]

- Diorio D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci.* 1993; 13:3839–3847. [PubMed: 8396170]
- Domenici MR, Azad SC, Marsicano G, Schierloh A, Wotjak CT, Dodt HU, Zieglgansberger W, Lutz B, Rammes G. Cannabinoid receptor type 1 located on presynaptic terminals of principal neurons in the forebrain controls glutamatergic synaptic transmission. *J Neurosci.* 2006; 26:5794–5799. [PubMed: 16723537]
- Doyon C, Denis RG, Baraboi ED, Samson P, Lalonde J, Deshaies Y, Richard D. Effects of rimonabant (SR141716) on fasting-induced hypothalamic-pituitary-adrenal axis and neuronal activation in lean and obese Zucker rats. *Diabetes.* 2006; 55:3403–3410. [PubMed: 17130486]
- Evanson NK, Tasker JG, Hill MN, Hillard CJ, Herman JP. Fast Feedback Inhibition of the HPA Axis by Glucocorticoids Is Mediated by Endocannabinoid Signaling. *Endocrinology.* 2010; 151:4811–4819. [PubMed: 20702575]
- Feldman S, Conforti N, Siegel RA. Adrenocortical responses following limbic stimulation in rats with hypothalamic deafferentations. *Neuroendocrinology.* 1982; 35:205–211. [PubMed: 7133323]
- Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev.* 2003; 83:1017–1066. [PubMed: 12843414]
- Fride E, Suris R, Weidenfeld J, Mechoulam R. Differential response to acute and repeated stress in cannabinoid CB1 receptor knockout newborn and adult mice. *Behav Pharmacol.* 2005; 16:431–440. [PubMed: 16148448]
- Furay AR, Bruestle AE, Herman JP. The role of the forebrain glucocorticoid receptor in acute and chronic stress. *Endocrinology.* 2008; 149:5482–5490. [PubMed: 18617609]
- Ganon-Elazar E, Akirav I. Cannabinoid receptor activation in the basolateral amygdala blocks the effects of stress on the conditioning and extinction of inhibitory avoidance. *J Neurosci.* 2009; 29:11078–11088. [PubMed: 19741114]
- Gonzalez S, Fernandez-Ruiz J, Di Marzo V, Hernandez M, Arevalo C, Nicanor C, Cascio MG, Ambrosio E, Ramos JA. Behavioral and molecular changes elicited by acute administration of SR141716 to Delta9-tetrahydrocannabinol-tolerant rats: an experimental model of cannabinoid abstinence. *Drug Alcohol Depend.* 2004; 74:159–170. [PubMed: 15099659]
- Goodwin RS, Baumann MH, Gorelick DA, Schwilke E, Schwoppe DM, Darwin WD, Kelly DL, Schroeder JR, Ortemann-Renon C, Bonnet D, Huestis MA. CB1 - Cannabinoid Receptor Antagonist Effects on Cortisol in Cannabis-Dependent Men. *Am J Drug Alcohol Abuse.* 2011 in press.
- Gorzalka BB, Hill MN, Hillard CJ. Regulation of endocannabinoid signaling by stress: implications for stress-related affective disorders. *Neurosci Biobehav Rev.* 2008; 32:1152–1160. [PubMed: 18433869]
- Haller J, Varga B, Ledent C, Barna I, Freund TF. Context-dependent effects of CB1 cannabinoid gene disruption on anxiety-like and social behaviour in mice. *Eur J Neurosci.* 2004; 19:1906–1912. [PubMed: 15078564]
- Hariri AR, Gorka A, Hyde LW, Kimak M, Halder I, Ducci F, Ferrell RE, Goldman D, Manuck SB. Divergent effects of genetic variation in endocannabinoid signaling on human threat- and reward-related brain function. *Biol Psychiatry.* 2009; 66:9–16. [PubMed: 19103437]
- Henckens MJ, van Wingen GA, Joels M, Fernandez G. Time-dependent effects of corticosteroids on human amygdala processing. *J Neurosci.* 2010; 30:12725–12732. [PubMed: 20861377]
- Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci.* 1991; 11:563–583. [PubMed: 1992016]
- Herman JP, Mueller NK. Role of the ventral subiculum in stress integration. *Behav Brain Res.* 2006; 174:215–224. [PubMed: 16876265]
- Herman JP, Ostrander MM, Mueller NK, Figueiredo H. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Prog Neuropsychopharmacol Biol Psychiatry.* 2005; 29:1201–1213. [PubMed: 16271821]
- Hill MN, Gorzalka BB. Impairments in endocannabinoid signaling and depressive illness. *JAMA.* 2009; 301:1165–1166. [PubMed: 19293417]

- Hill MN, Karatsoreos IN, Hillard CJ, McEwen BS. Rapid elevations in limbic endocannabinoid content by glucocorticoid hormones in vivo. *Psychoneuroendocrinology*. 2010a; 35:1333–1338. [PubMed: 20399021]
- Hill MN, McEwen BS. Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. *Prog Neuropsychopharmacol Biol Psychiatry*. 2010; 34:791–797. [PubMed: 19903506]
- Hill MN, McEwen BS. Endocannabinoids: The silent partner of glucocorticoids in the synapse. *Proc Natl Acad Sci U S A*. 2009; 106:4579–4580. [PubMed: 19293387]
- Hill MN, McLaughlin RJ, Bingham B, Shrestha L, Lee TT, Gray JM, Hillard CJ, Gorzalka BB, Viau V. Endogenous cannabinoid signaling is essential for stress adaptation. *Proc Natl Acad Sci U S A*. 2010b; 107:9406–9411. [PubMed: 20439721]
- Hill MN, McLaughlin RJ, Morrish AC, Viau V, Floresco SB, Hillard CJ, Gorzalka BB. Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. *Neuropsychopharmacology*. 2009a; 34:2733–2745. [PubMed: 19710634]
- Hill MN, McLaughlin RJ, Pan B, Fitzgerald ML, Roberts CJ, Lee TT, Karatsoreos IN, Mackie K, Viau V, Pickel VM, McEwen BS, Liu QS, Gorzalka BB, Hillard CJ. Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. *J Neurosci*. 2011; 31:10506–10515. [PubMed: 21775596]
- Hill MN, Miller GE, Carrier EJ, Gorzalka BB, Hillard CJ. Circulating endocannabinoids and N-acyl ethanolamines are differentially regulated in major depression and following exposure to social stress. *Psychoneuroendocrinology*. 2009b; 34:1257–1262. [PubMed: 19394765]
- Hill MN, Patel S, Campolongo P, Tasker JG, Wotjak CT, Bains JS. Functional interactions between stress and the endocannabinoid system: from synaptic signaling to behavioral output. *J Neurosci*. 2010c; 30:14980–14986. [PubMed: 21068301]
- Hillard CJ. Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. *Prostaglandins Other Lipid Mediat*. 2000; 61:3–18. [PubMed: 10785538]
- Hillard CJ, Weinlander KM, Stuhr KL. Contributions of endocannabinoid signaling to psychiatric disorders in humans: Genetic and biochemical evidence. *Neuroscience*. 2011 in press.
- Holsboer F. The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology*. 2000; 23:477–501. [PubMed: 11027914]
- Hu W, Zhang M, Czeh B, Flugge G, Zhang W. Stress impairs GABAergic network function in the hippocampus by activating nongenomic glucocorticoid receptors and affecting the integrity of the parvalbumin-expressing neuronal network. *Neuropsychopharmacology*. 2010; 35:1693–1707. [PubMed: 20357756]
- Jacobson L, Sapolsky R. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. *Endocr Rev*. 1991; 12:118–134. [PubMed: 2070776]
- Jaferi A, Bhatnagar S. Corticosterone can act at the posterior paraventricular thalamus to inhibit hypothalamic-pituitary-adrenal activity in animals that habituate to repeated stress. *Endocrinology*. 2006; 147:4917–4930. [PubMed: 16809449]
- Jaferi A, Nowak N, Bhatnagar S. Negative feedback functions in chronically stressed rats: role of the posterior paraventricular thalamus. *Physiol Behav*. 2003; 78:365–373. [PubMed: 12676271]
- Jones MT, Hillhouse EW, Burden JL. Structure-activity relationships of corticosteroid feedback at the hypothalamic level. *J Endocrinol*. 1977; 74:415–424. [PubMed: 303680]
- Karst H, Berger S, Erdmann G, Schutz G, Joels M. Metaplasticity of amygdalar responses to the stress hormone corticosterone. *Proc Natl Acad Sci U S A*. 2010; 107:14449–14454. [PubMed: 20663957]
- Karst H, Berger S, Turiault M, Tronche F, Schutz G, Joels M. Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. *Proc Natl Acad Sci U S A*. 2005; 102:19204–19207. [PubMed: 16361444]
- Katona I, Rancz EA, Acsady L, Ledent C, Mackie K, Hajos N, Freund TF. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J Neurosci*. 2001; 21:9506–9518. [PubMed: 11717385]

- Keim SR, Shekhar A. The effects of GABAA receptor blockade in the dorsomedial hypothalamic nucleus on corticotrophin (ACTH) and corticosterone secretion in male rats. *Brain Res.* 1996; 739:46–51. [PubMed: 8955923]
- Keller-Wood ME, Dallman MF. Corticosteroid inhibition of ACTH secretion. *Endocr Rev.* 1984; 5:1–24. [PubMed: 6323158]
- Kerr DM, Burke NN, Ford GK, Connor TJ, Harhen B, Egan LJ, Finn DP. Pharmacological inhibition of endocannabinoid degradation the expression of inflammatory mediators in the hypothalamus following an immunological stressor. *Neuroscience.* 2011 in press.
- Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science.* 1997; 277:1659–1662. [PubMed: 9287218]
- Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou Z, Huang BX, Kim HY, Kunos G. A biosynthetic pathway for anandamide. *Proc Natl Acad Sci U S A.* 2006; 103:13345–13350. [PubMed: 16938887]
- Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, Schlosburg JE, Pavon FJ, Serrano AM, Selley DE, Parsons LH, Lichtman AH, Cravatt BF. Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat Chem Biol.* 2009a; 5:37–44. [PubMed: 19029917]
- Long JZ, Nomura DK, Vann RE, Walentiny DM, Booker L, Jin X, Burston JJ, Sim-Selley LJ, Lichtman AH, Wiley JL, Cravatt BF. Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. *Proc Natl Acad Sci U S A.* 2009b; 106:20270–20275. [PubMed: 19918051]
- Malcher-Lopes R, Di S, Marcheselli VS, Weng FJ, Stuart CT, Bazan NG, Tasker JG. Opposing crosstalk between leptin and glucocorticoids rapidly modulates synaptic excitation via endocannabinoid release. *J Neurosci.* 2006; 26:6643–6650. [PubMed: 16775153]
- Manzanares J, Corchero J, Fuentes JA. Opioid and cannabinoid receptor-mediated regulation of the increase in adrenocorticotropin hormone and corticosterone plasma concentrations induced by central administration of delta(9)-tetrahydrocannabinol in rats. *Brain Res.* 1999; 839:173–179. [PubMed: 10482810]
- Marsicano G, Lutz B. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur J Neurosci.* 1999; 11:4213–4225. [PubMed: 10594647]
- Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature.* 1990; 346:561–564. [PubMed: 2165569]
- McDonald AJ, Mascagni F. Localization of the CB1 type cannabinoid receptor in the rat basolateral amygdala: high concentrations in a subpopulation of cholecystokinin-containing interneurons. *Neuroscience.* 2001; 107:641–652. [PubMed: 11720787]
- Mizoguchi K, Ishige A, Aburada M, Tabira T. Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. *Neuroscience.* 2003; 119:887–897. [PubMed: 12809708]
- Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993; 365:61–65. [PubMed: 7689702]
- Newsom RJ, Osterlund C, Masini CV, Day HE, Spencer RL, Campeau S. Cannabinoid receptor type 1 antagonism significantly modulates basal and loud noise-induced neural and HPA axis responses in male Sprague Dawley rats. *Neuroscience.* 2011 in press.
- Okamoto Y, Morishita J, Tsuboi K, Tonai T, Ueda N. Molecular characterization of a phospholipase D generating anandamide and its congeners. *J Biol Chem.* 2004; 279:5298–5305. [PubMed: 14634025]
- Onaivi ES, Ishiguro H, Gu S, Liu QR. CNS effects of CB2 cannabinoid receptors: beyond neuro-immuno-cannabinoid activity. *J Psychopharmacol.* 2011 in press.
- Pagotto U, Marsicano G, Fezza F, Theodoropoulou M, Grubler Y, Stalla J, Arzberger T, Milone A, Losa M, Di Marzo V, Lutz B, Stalla GK. Normal human pituitary gland and pituitary adenomas express cannabinoid receptor type 1 and synthesize endogenous cannabinoids: first evidence for a direct role of cannabinoids on hormone modulation at the human pituitary level. *J Clin Endocrinol Metab.* 2001; 86:2687–2696. [PubMed: 11397872]

- Patel KD, Davison JS, Pittman QJ, Sharkey KA. Cannabinoid CB(2) receptors in health and disease. *Curr Med Chem*. 2010; 17:1393–1410. [PubMed: 20166925]
- Patel S, Cravatt BF, Hillard CJ. Synergistic interactions between cannabinoids and environmental stress in the activation of the central amygdala. *Neuropsychopharmacology*. 2005a; 30:497–507. [PubMed: 15280883]
- Patel S, Roelke CT, Rademacher DJ, Cullinan WE, Hillard CJ. Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary-adrenal axis. *Endocrinology*. 2004; 145:5431–5438. 2004. [PubMed: 15331569]
- Patel S, Roelke CT, Rademacher DJ, Hillard CJ. Inhibition of restraint stress-induced neural and behavioural activation by endogenous cannabinoid signalling. *Eur J Neurosci*. 2005b; 21:1057–1069. [PubMed: 15787710]
- Pecoraro N, Dallman MF, Warne JP, Ginsberg AB, Laugero KD, la Fleur SE, Houshyar H, Gomez F, Bhargava A, Akana SF. From Malthus to motive: how the HPA axis engineers the phenotype, yoking needs to wants. *Prog Neurobiol*. 2006; 79:247–340. [PubMed: 16982128]
- Pertwee RG. Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. *Addict Biol*. 2008; 13:147–159. [PubMed: 18482430]
- Rademacher DJ, Meier SE, Shi L, Ho WS, Jarrahian A, Hillard CJ. Effects of acute and repeated restraint stress on endocannabinoid content in the amygdala, ventral striatum, and medial prefrontal cortex in mice. *Neuropharmacology*. 2008; 54:108–116. [PubMed: 17675104]
- Radley JJ, Arias CM, Sawchenko PE. Regional differentiation of the medial prefrontal cortex in regulating adaptive responses to acute emotional stress. *J Neurosci*. 2006; 26:12967–12976. [PubMed: 17167086]
- Radley JJ, Sawchenko PE. A common substrate for prefrontal and hippocampal inhibition of the neuroendocrine stress response. *J Neurosci*. 2011; 31:9683–9695. [PubMed: 21715634]
- Russell GM, Henley DE, Leendertz J, Douthwaite JA, Wood SA, Stevens A, Woltersdorf WW, Peeters BW, Ruigt GS, White A, Veldhuis JD, Lightman SL. Rapid glucocorticoid receptor-mediated inhibition of hypothalamic-pituitary-adrenal ultradian activity in healthy males. *J Neurosci*. 2010; 30:6106–6115. [PubMed: 20427668]
- Sapolsky RM, Krey LC, McEwen BS. Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. *Proc Natl Acad Sci U S A*. 1984; 81:6174–6177. [PubMed: 6592609]
- Shrestha, LD.; Hillard, CJ. Glucocorticoid effects on neuronal monoacylglycerol lipase activity; 20th Annual Symposium of the International Cannabinoid Research Society; 2010; p. 3-18.
- Simon GM, Cravatt BF. Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. *J Biol Chem*. 2006; 281:26465–26472. [PubMed: 16818490]
- Singewald GM, Rjabokon A, Singewald N, Ebner K. The modulatory role of the lateral septum on neuroendocrine and behavioral stress responses. *Neuropsychopharmacology*. 2011; 36:793–804. [PubMed: 21160468]
- Singh ME, Verty AN, Price I, McGregor IS, Mallet PE. Modulation of morphine-induced Fos-immunoreactivity by the cannabinoid receptor antagonist SR 141716. *Neuropharmacology*. 2004; 47:1157–1169. [PubMed: 15567425]
- Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. *Proc Natl Acad Sci U S A*. 2002; 99:8394–8399. [PubMed: 12060782]
- Sipe JC, Scott TM, Murray S, Harismendy O, Simon GM, Cravatt BF, Waalen J. Biomarkers of endocannabinoid system activation in severe obesity. *PLoS One*. 2010; 5:e8792. [PubMed: 20098695]
- Spencer SJ, Buller KM, Day TA. Medial prefrontal cortex control of the paraventricular hypothalamic nucleus response to psychological stress: possible role of the bed nucleus of the stria terminalis. *J Comp Neurol*. 2005; 481:363–376. [PubMed: 15593338]
- Steiner MA, Marsicano G, Nestler EJ, Holsboer F, Lutz B, Wotjak CT. Antidepressant-like behavioral effects of impaired cannabinoid receptor type 1 signaling coincide with exaggerated corticosterone secretion in mice. *Psychoneuroendocrinology*. 2008; 33:54–67. [PubMed: 17976922]

- Stetler C, Miller GE. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. *Psychosom Med*. 2011; 73:114–126. [PubMed: 21257974]
- Sugiura T, Kobayashi Y, Oka S, Waku K. Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological significance. *Prostaglandins Leukot Essent Fatty Acids*. 2002; 66:173–192. [PubMed: 12052034]
- Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun*. 1995; 215:89–97. [PubMed: 7575630]
- Tasker JG, Herman JP. Mechanisms of rapid glucocorticoid feedback inhibition of the hypothalamic-pituitary-adrenal axis. *Stress*. 2011; 14:398–406. [PubMed: 21663538]
- Ueda N. Endocannabinoid hydrolases. *Prostaglandins Other Lipid Mediat*. 2002; 68-69:521–534. [PubMed: 12432941]
- Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci*. 2009; 10:397–409. [PubMed: 19469025]
- Uriguen L, Perez-Rial S, Ledent C, Palomo T, Manzanares J. Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. *Neuropharmacology*. 2004; 46:966–973. [PubMed: 15081793]
- Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Marnett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA. Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science*. 2005; 310:329–332. [PubMed: 16224028]
- Viau V, Meaney MJ. The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial preoptic area. *J Neurosci*. 1996; 16:1866–1876. [PubMed: 8774455]
- Wade MR, Degroot A, Nomikos GG. Cannabinoid CB1 receptor antagonism modulates plasma corticosterone in rodents. *Eur J Pharmacol*. 2006; 551:162–167. [PubMed: 17030030]
- Wamsteeker JI, Kuzmiski JB, Bains JS. Repeated stress impairs endocannabinoid signaling in the paraventricular nucleus of the hypothalamus. *J Neurosci*. 2010; 30:11188–11196. [PubMed: 20720126]
- Wang M, Hill MN, Zhang L, Gorzalka BB, Hillard CJ, Alger BE. Acute restraint stress enhances hippocampal endocannabinoid function via glucocorticoid receptor activation. *J Psychopharmacol*. 2011 in press.
- Wilson RI, Nicoll RA. Endocannabinoid signaling in the brain. *Science*. 2002; 296:678–682. [PubMed: 11976437]
- Xi ZX, Peng XQ, Li X, Song R, Zhang HY, Liu QR, Yang HJ, Bi GH, Li J, Gardner EL. Brain cannabinoid CB(2) receptors modulate cocaine's actions in mice. *Nat Neurosci*. 2011; 14:1160–1166. [PubMed: 21785434]
- Yuen EY, Liu W, Karatsoreos IN, Feng J, McEwen BS, Yan Z. Acute stress enhances glutamatergic transmission in prefrontal cortex and facilitates working memory. *Proc Natl Acad Sci U S A*. 2009; 106:14075–14079. [PubMed: 19666502]
- Ziegler CG, Mohn C, Lamounier-Zepter V, Rettori V, Bornstein SR, Krug AW, Ehrhart-Bornstein M. Expression and function of endocannabinoid receptors in the human adrenal cortex. *Horm Metab Res*. 2010; 42:88–92. [PubMed: 19862666]

Figure 1.

Under basal conditions (left panel), there is an anandamide (AEA) tone within the basolateral nucleus of the amygdala (BLA) which gates the presynaptic release of glutamate, through activation of CB₁ receptors on glutamatergic terminals, and thus constrains the excitability of projection neurons of the BLA. In response to stress (right panel), hydrolysis of AEA by fatty acid amide hydrolase (FAAH) increases reducing signalling levels of AEA. This loss of AEA signalling at the CB₁ receptor disinhibits glutamatergic inputs to projection neurons of the BLA, resulting in an increase in the firing activity and outflow of BLA projection neurons. Increased activation of the amygdala will contribute to activation of a stress response, which ultimately increases activation of the HPA axis.

Figure 2.

(1) Exposure to stress causes activation of CRH neurosecretory cells within the paraventricular nucleus of the hypothalamus (PVN); (2) CRH stimulates the release of ACTH into the general circulation where it acts on the adrenal gland to induce the synthesis and release of glucocorticoid hormones; (3) Glucocorticoid hormones enter the circulation and penetrate the brain where they activate negative feedback pathways; (4) Within the PVN glucocorticoids cause a rapid release of endocannabinoids which suppress excitatory inputs to the CRH cells in the PVN to contribute to fast-feedback inhibition of the HPA axis. Within the prefrontal cortex (PFC), glucocorticoids cause a delayed increase in endocannabinoid mobilization, which suppresses local inhibitory circuits and increases the outflow of projection neurons from the PFC. (5) These projection neurons from the PFC activate inhibitory relay neurons to the PVN to turn off neuronal activation of the PVN and contribute to delayed glucocorticoid negative feedback of the HPA axis. Combined, the recruitment of endocannabinoid signalling acts at both short (PVN) and long (PFC) loops of the negative feedback pathway in the brain.

Figure 3.

AEA and 2-AG exhibit a ying-yang relationship with HPA axis activity. Under basal conditions, AEA tonically suppresses HPA axis activity. In response to stress, AEA levels rapidly decline within the amygdala, disinhibiting HPA axis activity and resulting in an increase in glucocorticoid hormone secretion. Glucocorticoid hormones act to increase 2-AG production, which then acts to suppress HPA axis activity through actions in both the hypothalamus (rapid) and prefrontal cortex (delayed). In addition, glucocorticoids also act to normalize AEA levels within the amygdala, and thus remove the disinhibition on the HPA axis and help return HPA function to basal levels.