



Published in final edited form as:

*Prog Neuropsychopharmacol Biol Psychiatry*. 2010 June 30; 34(5): 791–797. doi:10.1016/j.pnpbp.2009.11.001.

## Involvement of the Endocannabinoid System in the Neurobehavioral Effects of Stress and Glucocorticoids

**Matthew N. Hill** and **Bruce S. McEwen**

Laboratory of Neuroendocrinology, Rockefeller University, New York, NY USA 10065

### Abstract

The endocannabinoid system is a neuroactive lipid signaling system that functions to gate synaptic transmitter release. Accumulating evidence has demonstrated that this system is responsive to modulation by both stress and glucocorticoids within the hypothalamus and limbic structures; however, the nature of this regulation is more complex than initially assumed. The aim of the current review is to summarize the research to date which examines the effects of acute stress and glucocorticoid administration on endocannabinoid signaling in limbic-hypothalamic-pituitary-adrenal (LHPA), and in turn the role endocannabinoid signaling plays in the neurobehavioral responses to acute stress and glucocorticoid administration. The majority of research suggests that acute stress produces a mobilization of the endocannabinoid 2-arachidonoylglycerol (2-AG) while concurrently reducing the tissue content of the other endocannabinoid ligand anandamide. Genetic and pharmacological studies demonstrate that the reduction in anandamide signaling may be involved in the initiation of HPA axis activation and the generation of changes in emotional behaviour, while the increase in 2-AG signaling may be involved in terminating the stress response, limiting neuronal activation and contributing to changes in motivated behaviours. Collectively, these studies reveal a complex interplay between endocannabinoids and the HPA axis, and further identify endocannabinoid signaling as a critical regulator of the stress response.

### The Endocannabinoid System

The endocannabinoid system was first characterized as the neuronal system to which the psychoactive constituent of cannabis, delta-9-tetrahydrocannabinol (THC), interacted to exert its effects on physiology and behavior. The endocannabinoid system is a unique system, exerting modulatory actions in both central tissue and in the periphery. In the brain, endocannabinoids are generated “on demand” and act retrogradely to regulate release of neurotransmitters. At the signaling level, two cannabinoid receptors have been characterized to date (Howlett, 2002). The cannabinoid CB<sub>1</sub> receptor is the receptor that is expressed ubiquitously throughout most regions of the brain (Herkenham et al., 1991; Moldrich and Wenger, 2000; Tsou et al., 1998); however, the CB<sub>1</sub> receptor is also known to exhibit some expression patterns in peripheral tissue, such as immune cells, vascular tissue and adipocytes (Cota et al., 2003; Hillard, 2000; Parolaro, 1999). While the CB<sub>2</sub> receptor is located predominately in peripheral immune cells and organs (Munro et al., 1993), CB<sub>2</sub> receptors are also expressed by microglial cells in injured, infected or inflamed CNS tissue (Benito et al., 2008). There is also recent evidence that cannabinoid CB<sub>2</sub> receptors exhibit limited

Correspondence to be directed to: Matthew N. Hill, Ph.D., Laboratory of Neuroendocrinology, The Rockefeller University, 1230 York Avenue, Box 165, New York, NY USA 10065, Tel: 212-327-8623; Fax: 212-327-8634; mhill@rockefeller.edu.

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

neuronal expression (Van Sickle et al., 2005) and are present in neural progenitor cells within the hippocampus (Palazuelos et al., 2006).

Both CB<sub>1</sub> and CB<sub>2</sub> receptors are G-protein coupled receptors. The CB<sub>1</sub> receptor couples to both G<sub>i/o</sub> proteins which function to inhibit adenylyl cyclase activity, activate potassium channels and inhibit voltage-gated calcium channels, while the CB<sub>2</sub> receptor is only known to couple to G<sub>i</sub> proteins (Howlett, 2002). The CB<sub>1</sub> receptor appears to be located predominately on presynaptic axon terminals, and is capable of regulating calcium influx, and hence neurotransmitter release. Evidence shows that the endocannabinoid system has the ability to inhibit glutamate, GABA, acetylcholine, serotonin and norepinephrine release (Freund et al., 2003; Schlicker and Kathmann, 2001).

The endogenous ligands for cannabinoid receptors are the arachidonate derived lipophilic molecules N-arachidonyl ethanolamine (anandamide; AEA (Devane et al., 1992)) and 2-arachidonylglycerol (2-AG (Sugiura et al., 1995)). Both AEA and 2-AG do not behave as typical neurotransmitters. It is currently believed that both AEA and 2-AG are formed postsynaptically by activity-dependent cleavage of phospholipids' head groups by activation of specific enzymes, although activity-independent mechanisms of endocannabinoid synthesis have also been demonstrated. The biosynthesis of 2-AG is mediated by 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 and Waku, 2002). The pathways mediating AEA synthesis are less well understood. To date, three distinct and independent mechanisms have been found to generate AEA (Liu et al., 2006; Okamoto et al., 2004; 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)

Endocannabinoids are believed to be formed in post-synaptic cells by excitatory activity and are released into the synapse where they act in a retrograde manner to activate their presynaptically located receptor and inhibit neurotransmitter release (Ohno-Shosaku et al., 2001; Schlicker and Kathmann, 2001; Wilson et al., 2001). Termination of endocannabinoid signaling is determined by metabolic enzymes. 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 (Deutsch et al., 2002; Dinh et al., 2002; Ueda, 2002).

## **Stress, the HPA Axis and the Regulation of Endocannabinoid Signaling**

Stress is typically defined as any stimulus that represents a perceived or actual threat to homeostatic functioning. The most common physiological response to stressful stimuli is the activation of the hypothalamic-pituitary-adrenal (HPA) axis which governs the neuroendocrine response to aversive stimuli. Activation of corticotropin releasing hormone (CRH) neurosecretory cells within the paraventricular nucleus of the hypothalamus (PVN) is the initiating step of the adrenocortical response to stress; the endpoint of which is the release of glucocorticoid hormones (corticosterone in rodents and cortisol in humans) into the general circulation. Glucocorticoids, in turn, promote glucose mobilization and redirect energy stores necessary for rapid, adaptive responses to stress (Pecoraro et al., 2006). At the central level, glucocorticoids can either mediate some of the responses to stress (e.g., activation of glutamate transmission in the hippocampus; (Karst et al., 2005)) or act to promote reinstatement in a system following a disturbance elicited by the stressful stimuli (e.g., suppression of CRH induction evoked by stressful stimuli; (Keller-Wood and Dallman, 1984)).

The primary means of HPA axis regulation occurs through a well characterized negative feedback loop in which glucocorticoids suppress ongoing HPA axis activity at both the hypothalamic and pituitary level (Pecoraro et al., 2006). However, HPA axis regulation is more complex than a thermostat model in which circulating glucocorticoids dictate the steady state activity of the HPA axis. For example, in the absence of glucocorticoids (following adrenalectomy), consumption of sucrose can normalize increased CRH expression and ACTH secretion (Laugero et al., 2001) indicating the presence of mechanisms of HPA axis regulation beyond glucocorticoid negative feedback.

With respect to extrahypothalamic regulation of the HPA axis, lesion studies have clearly demonstrated the importance of both forebrain and hindbrain regions for regulation of the HPA axis under both basal and stress conditions (Herman et al., 2003; Pecoraro et al., 2006). In response to stress, these regions appear to be differentially involved in activation of the HPA axis such that neural inputs to the PVN that originate from hindbrain structures are involved in activating the HPA axis following a physiological stressor (e.g., internal, physiological disturbances in homeostasis, such as fluid loss or immune challenge (Herman et al., 2003; Pecoraro et al., 2006)), while those originating in the forebrain are involved in HPA axis activation following psychological stressors (e.g., external, psychological disturbances, such as threat signals (Ulrich-Lai and Herman, 2009)). Taken together, the HPA axis is an intricate system which exhibits both simple and complex means of regulation and exerts both rapid and delayed effects on neuronal function.

With respect to the endocannabinoid system, the first studies to suggest that endocannabinoid signaling may be regulated by stress or glucocorticoids came from *in vitro* research from the laboratory of Jeffrey Tasker. Through a series of elegant studies, Tasker and colleagues demonstrated that within the PVN of the hypothalamus (as well as the supraoptic nucleus), glucocorticoids evoked a rapid induction of endocannabinoid synthesis and release through a non-genomic mechanism involving a G<sub>s</sub> coupled membrane bound receptor (Di et al., 2005a; Di et al., 2003; Di et al., 2005b; Di et al., 2009; Malcher-Lopes et al., 2006). This discovery helped to clarify a long-standing set of findings of a G-protein coupled glucocorticoid receptor in the newt, *Taricha granulosa* (Orchinik et al., 1992; Orchinik et al., 1991). The glucocorticoid-mediated release of endocannabinoids within the PVN, in turn, resulted in the suppression of incoming excitatory neurotransmission to CRH neurosecretory cells and provided a mechanism by which glucocorticoids could exert rapid shut down of the HPA axis (Di et al., 2003). Two broader implications of these findings were that: 1) endocannabinoid signaling was capable of dampening HPA axis activity, and 2) endocannabinoid signaling within the hypothalamus could be induced by stress and glucocorticoids.

At the *in vivo* level, the first study to examine the effects of stress on endocannabinoid signaling was performed in the laboratory of Cecilia Hillard, in which, contrary to expectations, acute stress to mice was found to result in a reduction in 2-AG content within the hypothalamus without affecting the tissue levels of AEA (Patel et al., 2004). Examination of the effects of acute restraint stress on other brain structures revealed that there was no effect of acute stress on endocannabinoid ligand content in the forebrain or cerebellum, and within the amygdala stress resulted in a reduction AEA content without affecting 2-AG levels (Patel et al., 2005). Subsequent studies from this same group examining the effects of a common acute restraint stress on endocannabinoid content in the mouse found similar results in that stress did not modulate endocannabinoid content in the medial prefrontal cortex (PFC) or ventral striatum, but again produced a reduction in amygdalar AEA content without affecting 2-AG (Rademacher et al., 2008). As such, these studies were not very consistent with the *in vitro* work performed by Tasker and colleagues.

Interestingly, subsequent studies employing rats instead of mice (which is the species employed by Tasker and colleagues for their *in vitro* work), found quite different results. An initial study from the group of Andrea Hohmann found that brief exposure to a foot shock stress resulted in a dramatic and rapid elevation in both AEA and 2-AG levels within the periaqueductal grey (Hohmann et al., 2005). Additional studies using the model of acute restraint stress in rats produced results more consistent with those seen by Tasker and colleagues, than what was seen in the studies employing mice. Specifically, 30 minute exposure to restraint stress in rats was found to produce an elevation in 2-AG levels in the PFC, hippocampus and hypothalamus (Hill et al., 2007), but not within the amygdala (Hill et al., 2009c). Interestingly, AEA content was correspondingly found to be reduced by stress within the PFC (Hill et al., 2007) hippocampus (Hill et al., 2007) and the amygdala (Hill et al., 2009c), but not the hypothalamus (Hill et al., 2007). This reduction in AEA, at least within the amygdala, does appear to be due, in part, to a rapid induction of FAAH activity as 30 min of restraint stress was found to increase FAAH activity 3-fold within the amygdala (Hill et al., 2009c).

Thus, a pronounced species difference appears to be present between the effects of stress on endocannabinoid signaling in mice and rats. In rats, stress appears to mobilize 2-AG signaling, while suppressing AEA signaling, in a variety of limbic structures. In mice, there does not appear to be an effect of stress on 2-AG (at least through the examination of tissue levels of endocannabinoid content), while the reduction in AEA still seems to be present. The effects of acute stress on central endocannabinoid content can be seen in Table 1. Interestingly, while no studies have been performed on central endocannabinoid content following stress exposure in humans, we have recently reported that exposure of women to the Trier social stress test results in a rapid increase in circulating levels of 2-AG while not affecting circulating levels of AEA (Hill et al., 2009d), suggesting that stress-induced mobilization of 2-AG is a conserved mechanism from rats to humans

To further determine if these effects of acute stress are mediated by glucocorticoid secretion, we subsequently examined the effects of glucocorticoid administration (in the absence of a stressor) on endocannabinoid content within these same limbic structures. Much to our surprise, a single administration of corticosterone was found to robustly increase AEA content within the amygdala, hippocampus and hypothalamus at only 10 min following administration (Hill et al., 2009b). 2-AG content was also found to be elevated 10 minutes following corticosterone administration, but only within the hypothalamus (Hill et al., 2009b). At 1 hour following administration of corticosterone, these effects had almost entirely subsided (Hill et al., 2009b). These data corresponded quite well with the *in vitro* findings of Tasker and colleagues and demonstrated that *in vivo*, glucocorticoids (likely acting through a non-genomic mechanism) evoked a rapid induction of endocannabinoid signaling within limbic structures in rats.

The fact that stress increases 2-AG and decreases AEA, while corticosterone predominately increases AEA, with 2-AG elevations only being detected in the hypothalamus, suggests that the effects of stress and glucocorticoids on 2-AG and AEA content are likely mediated through distinct mechanisms. With respect to AEA, one possibility is that stress (through changes in neurotransmission or excitability that precedes activation of the HPA axis) decreases AEA content, and that glucocorticoids increase AEA content in an attempt to normalize AEA signaling following stress. This hypothesis is in line with the role of glucocorticoids to reinstate homeostasis in circuits that have been disrupted by stress.

With respect to 2-AG, both stress and glucocorticoids do appear to increase 2-AG (at least in rats); however, the effects produced by stress are more robust than those seen following administration of glucocorticoids alone. Thus, it is possible that glucocorticoids are capable

of inducing 2-AG synthesis, but that perhaps the coordination of glucocorticoids and neuronal activation (producing elevations in post synaptic calcium conductance) induced by stress produce optimal conditions that mobilize 2-AG greater than following glucocorticoid administration alone. Future studies involving the removal of adrenal steroids will be required to conclusively determine the factors driving changes in endocannabinoid content following stress.

## Functional Role of Endocannabinoid Signaling in the Neurobehavioral Effects of Acute Stress

Collectively, the current level of knowledge suggests that exposure to acute stress mobilizes 2-AG, while decreasing AEA, in limbic structures. Through the use of pharmacological and genetic manipulations, several studies have attempted to unmask the relevance of the induction of 2-AG signaling following stress to determine if an increase in endocannabinoid signaling is contributing to the neurobehavioral effects of stress.

With respect to the neuroendocrine response that occurs in response to stress, genetic or pharmacological blockade of the CB<sub>1</sub> receptor has consistently demonstrated that disruption of this system exaggerates the neuroendocrine response to stress, suggesting that endocannabinoid signaling in the hypothalamus limits responsivity of the HPA axis to stress. Specifically, mice lacking the CB<sub>1</sub> receptor have been found to exhibit potentiated secretion of both ACTH and corticosterone following exposure to an array of psychological stressors such as restraint (Uriguen et al., 2004), tail suspension (Aso et al., 2008), forced swim (Steiner et al., 2008) and novelty stress (Barna et al., 2004; Haller et al., 2004). Similarly, pharmacological antagonism of the CB<sub>1</sub> receptor potentiates stress-induced glucocorticoid secretion and neuronal activation within the PVN (Patel et al., 2004; Steiner et al., 2008). These data suggest that endocannabinoid signaling is engaged by stress to constrain activation of the HPA axis; if this signaling is disrupted an exaggerated response occurs.

One possible mechanism to account for this would be a disruption of glucocorticoid mediated rapid inhibition of the HPA axis. Specifically, once glucocorticoids are secreted in response to stress, they exert a rapid feedback inhibition of the HPA axis, possibly by engaging endocannabinoid signaling to suppress excitatory transmission which activates CRH neurosecretory cells in the PVN. By blocking the induction of endocannabinoid signaling elicited by glucocorticoids, it is possible that this prevents the early phase of HPA axis shutdown by glucocorticoids and results in an exaggerated activation of the HPA axis. In support of this hypothesis, recent data has demonstrated that local inhibition of CB<sub>1</sub> receptors within the PVN can impair glucocorticoid-mediated rapid inhibition of the HPA axis (Evanson et al., 2007). Thus, one functional role of an induction of 2-AG signaling in the hypothalamus in response to stress may be to limit HPA axis activation and contribute to termination of the stress response. These data indicate that the endocannabinoid system is involved in the “thermostat” regulation of the HPA axis, but it does not preclude that endocannabinoid signaling also contributes to extrahypothalamic regulation of the HPA axis. Further research is required to fully understand the nature of the endocannabinoid system in the regulation of the HPA axis under conditions of stress.

Similar to the hypothesis that induction of endocannabinoid signaling in response to stress may limit activation of the HPA axis, there is also evidence that endocannabinoid signaling may constrain activation of specific neuronal populations in response to stress. Using the immediate early gene *c-fos* as a marker of neuronal activation, it has been found that pharmacological antagonism of the CB<sub>1</sub> receptor can potentiate stress-induced neuronal activation in a subset of forebrain regions such as the cingulate cortex, lateral septum and the nucleus accumbens (Patel et al., 2005). This would suggest that in response to stress, an

induction of endocannabinoid signaling curbs neuronal activation in these structures, possibly through a regulation of glutamate release (Patel et al., 2005). Similarly, microdialysis studies have found that the ability of stress to promote acetylcholine release in the hippocampus is facilitated in mice lacking the CB<sub>1</sub> receptor (despite no differences in basal acetylcholine levels; (Degroot et al., 2006)). These data indicate that a mobilization of endocannabinoid signaling in response to stress functions to limit transmitter release and neuronal activation in limbic structures.

At the behavioural level, endocannabinoids have been experimentally determined to mediate the effects of stress on two behavioural processes. One common behavioural response to stress in animals and humans is an increase in the consumption of addictive substances or a relapse following a period of abstinence. In mice which have had several weeks access to an ethanol solution, exposure to a foot shock stress resulted in a dramatic increase in ethanol consumption over the following 24 h period following stress (Racz et al., 2003); in mice lacking the CB<sub>1</sub> receptor, stress did not promote ethanol consumption (Racz et al., 2003). This data suggest that stress-induced mobilization of endocannabinoid signaling mediates an increase in ethanol consumption, a finding which is consistent with the fact that facilitation of endocannabinoid neurotransmission, in and of itself, promotes ethanol consumption (Blednov et al., 2007; Hansson et al., 2007).

In addition to promotion of ethanol consumption, there is also evidence that endocannabinoids mediate the suppressive effects of stress on sexual behaviour. Stress is widely known to dampen sexual activity, a phenomenon that has been widely studied in the roughskin newt, *Taricha granulosa*, given the rapidity of onset and reliability of the effect (Rose and Moore, 1999). Using this model, it has been found that pharmacological blockade of the CB<sub>1</sub> receptor can prevent the inhibitory effects of stress exposure on male courtship behaviour, indicating mediation through an induction of endocannabinoid signaling (Coddington et al., 2007). This finding also agrees with the general inhibitory effect CB<sub>1</sub> receptor activation has been found to exert over male reproductive behaviours (Gorzalka et al., 2009). Collectively, these data provide the first experimental evidence to suggest that stress-induced mobilization of endocannabinoid signaling plays an important role in the regulation of neural, endocrine and behavioural responses to stress.

While the latter studies reveal that facilitation of endocannabinoid signaling in response to stress plays a functional role in some neurobehavioral responses to stress, they do not explain the functional consequences of the decline in limbic AEA levels that occurs following stress exposure. To examine the role of this change, studies have employed the use of pharmacological agents which inhibit the uptake and/or metabolism of AEA to prevent the stress-induced reduction in AEA content. Consistent with the effects of CB<sub>1</sub> receptor antagonism on stress-induced activation of the HPA axis, inhibition of AEA uptake and/or metabolism has been found to dampen stress-induced corticosterone secretion (Patel et al., 2004). Unlike the hypothesis that stress induces endocannabinoid signaling to promote feedback inhibition of the HPA axis, these data instead suggest that AEA signaling may exert a steady state regulation of the HPA axis, and that removal of this AEA tone in response to stress may permit activation of the HPA axis. In this sense, AEA signaling may function more as a “gatekeeper” (Patel et al., 2004). Interestingly, while the locus of action for CB<sub>1</sub> receptor antagonists to increase HPA axis activation appears to be within the PVN proper (Evanson et al., 2007), the HPA axis limiting effects of AEA appear to be due to an upstream site of action, in particular the basolateral amygdala (Hill et al., 2009c).

Within the basolateral amygdala, FAAH activity is found to be rapidly induced by stress, which results in a decline in AEA content within this structure (Hill et al., 2009c). The functional role of this decline, with respect to the HPA axis, was highlighted by the finding

that local administration of a FAAH inhibitor into the basolateral amygdala (but not the central or medial nuclei of the amygdala) attenuated activation of the HPA axis in response to stress (Hill et al., 2009c).

These data support the “gatekeeper” hypothesis of AEA regulation of the HPA axis, but add to this model by indicating that a reduction of AEA tone within the basolateral amygdala contributes to activation of the HPA axis (Hill et al., 2009c). The working theory of these findings is that under basal conditions AEA levels within the basolateral amygdala are high and provide a steady state constraint of excitatory transmission onto the projection neurons of the basolateral amygdala. In response to stress, FAAH activity increases and AEA levels decrease, which results in a disinhibition of excitation and increased firing activity of the projection neurons from the basolateral amygdala, which ultimately increases neuronal activity within the PVN and thus the HPA axis (Hill et al., 2009c). Thus, one functional consequence of the stress-induced decline in AEA signaling appears to removal of an inhibitory tone over the HPA axis. Taken with the hypothesis that stress-induced 2-AG signaling in the PVN may function to promote feedback inhibition on the HPA axis, these data create a ying-yang model of endocannabinoids and stress-induced HPA axis activation whereby endocannabinoid signaling is involved in both the activation and inhibition of the HPA axis in response to stress.

In addition to the role of a stress-induced decline in AEA signaling in regulation of the HPA axis, there is data to suggest that this reduction in AEA signaling may be involved in other effects of stress. Within the hippocampus, AEA signaling is known to promote cell proliferation as genetic deletion of FAAH has been found to significantly increase progenitor cell proliferation in the dentate gyrus (Aguado et al., 2005). As stress is known to produce a reduction in AEA signaling in this region, we sought to determine if this decline in AEA was involved in the reduction in cell proliferation that occurs in this region following exposure to stress. Our data demonstrate that administration of the AEA uptake inhibitor/FAAH inhibitor AM404 prior to stress exposure prevented the suppression of cell proliferation in the dentate gyrus following exposure to predator odor stress (Hill et al., 2006). These data indicate that one possible outcome of the reduction in AEA signaling following stress is a consequential reduction in cell proliferation in the hippocampus. It has yet to be determined if this decline in AEA following stress is involved in changes in other forms of neuroplasticity.

At the behavioural level, this decline in AEA also appears to relate to changes in emotional behaviour. Specifically, exposure to predator odor stress is known to produce a behavioural phenotype of defensive burying that is believed to represent an anxiety-like behaviour (McGregor et al., 2004). Administration of an AEA uptake/FAAH inhibitor prior to stress exposure significantly attenuates the induction of this burying behaviour in a similar manner to anxiolytic agents (Hill et al., 2006). These data would suggest that the decline in AEA signaling that occurs in response to stress contributes to the increase in emotional and anxiety-like behaviours that accompanies stress exposure. Interestingly, this hypothesis may help to explain the anxiolytic effects of FAAH inhibitors. Specifically, a series of studies has consistently demonstrated that inhibition of FAAH does not produce anxiolytic effects unless the environmental conditions in which testing occurs are particularly aversive (Haller et al., 2009; Naidu et al., 2007). This finding suggests that inhibition of FAAH under stable conditions does little to modulate emotional behaviour, but following exposure to aversive environmental conditions, FAAH activity may increase which results in a decline in AEA signaling which is conducive to the development of anxiety-like behaviours. As such, inhibition of FAAH may counter the decline in AEA that occurs following exposure to stressful stimuli, and in turn, prevent the facilitation of anxiety-like behaviour that accompanies stressful experiences. This hypothesis can account for both the

environmentally specific effects of FAAH inhibitors and the modest effects that FAAH inhibition produces on anxiety behaviour relative to a standard anxiolytic, such as diazepam (Haller et al., 2009; Naidu et al., 2007).

Collectively, these data create an emerging picture of differential roles of the endocannabinoid ligands AEA and 2-AG in the neurobehavioral responses to stress. An induction of 2-AG in response to stress throughout the forebrain may contribute to both constraining neuronal activation and HPA axis activity as well as drive behavioural changes such as ethanol consumption and suppression of sexual behaviour. On the other hand, a reduction in AEA signalling may disinhibit neuronal activity in limbic-hypothalamic structures and promote HPA axis activation and changes in emotional behaviour and neuroplasticity. While time course studies have not been properly performed in this regard, there may be a temporal nature to these changes such that the decline in AEA happens relatively rapidly following stress exposure to promote immediate responses to aversive environmental stimuli (increased HPA axis activity and emotional reactivity), while the increase in 2-AG occurs at a later time point and is more involved in the termination of the stress response and a return to homeostasis.

### **Functional Role of Endocannabinoids in the Acute Neurobehavioral Effects of Glucocorticoids**

As mentioned, glucocorticoid administration appears to exert similar, but distinct, regulation of endocannabinoid ligands as stress does. Thus while stress decreases AEA and increases 2-AG in most limbic brain structures, acute glucocorticoid administration rapidly increases AEA in most limbic regions while only exerting a rapid induction of 2-AG within the hypothalamus (Hill et al., 2009b). Our current working theory on these differences is that glucocorticoids, through actions at a membrane bound  $G_s$  coupled receptor, rapidly induce AEA synthesis. With respect to the relative absence of effects of systemically administered glucocorticoids on 2-AG content, it is possible that this non-genomic glucocorticoid receptor activation needs to be coupled to changes in neuronal activation and increased calcium conductance (as would be seen following stress) to induce noticeable changes in tissue levels of 2-AG. Thus, glucocorticoids may be necessary for the effects of acute stress on 2-AG induction, but for AEA they may be more important for reinstating AEA levels following the rapid decline that is evoked by stress. It should be noted, however, that this model is hypothetical and requires experimental validation.

Regardless of the differential effects that stress and glucocorticoids exert on the responses of both AEA and 2-AG, similar to stress, there is some evidence that endocannabinoid signalling is involved in the behavioural effects of glucocorticoids. As the rapid, non-genomic effects of glucocorticoids are a relatively unstudied field of research, two recent studies have revealed reliable, rapid effects of glucocorticoids that are dependent upon endocannabinoid signalling. Similar to the effects of stress, administration of corticosterone to the rough-skinned newt results in suppression of courtship behaviour that is blocked by a  $CB_1$  receptor antagonist (Coddington et al., 2007). Consistent with this behavioural response, administration of corticosterone suppressed neuronal activation within hindbrain sensory receptive neurons in a  $CB_1$  receptor antagonist sensitive manner (Coddington et al., 2007). These data indicate that administration of corticosterone rapidly induces endocannabinoid synthesis to modulate synaptic transmitter release and modulate behavioural responding.

While these data are quite compelling, they are limited by the fact that these studies are performed in an amphibian species which may behave physiologically different than a mammalian system. However, a recent study demonstrated the cross-species relevance of



this phenomenon by revealing that endocannabinoid signalling in the amygdala is involved in the effects of glucocorticoids on aversive memory consolidation (Campolongo et al., 2009). It has been previously reported that systemic administration of glucocorticoids can facilitate the consolidation of aversive memories through a rapid action in the amygdala that involves a reduction in GABAergic neurotransmission (Roosendaal et al., 2009). This study illustrated mediation of this phenomenon by endocannabinoid signalling, in that local administration of a CB<sub>1</sub> receptor antagonist into the basolateral amygdala immediately prior to administration of corticosterone mitigated that ability of corticosterone to facilitate aversive memory consolidation (Campolongo et al., 2009). Taken together with our recent biochemical findings, these data indicate that glucocorticoids (in the absence of stress) are capable of promoting endocannabinoid mobilization (likely AEA) within the amygdala to modulate local synaptic transmitter release and influence behavioural responses to environmental stimuli.

While the data presented here are sparse, they do collectively form a cohesive argument that glucocorticoids can promote endocannabinoid signalling through a non-genomic pathway to exert rapid behavioural changes. The role of endocannabinoids in other rapid behavioural effects of glucocorticoids such changes in aggressive behaviour (Mikics et al., 2004) and risk assessment (Mikics et al., 2005) should also be investigated to determine if endocannabinoid signalling is a broad mediator of the rapid behavioural effects of glucocorticoids.

## Summary and Conclusion

The aim of the current review was to summarize the state of knowledge regarding the regulation of endocannabinoid signalling by acute stress and glucocorticoids, and the functional role these changes may possess with respect to the neurobehavioral responses to stress and glucocorticoids. In addition to the data reviewed herein, there is a substantial body of research which has also demonstrated a complex role of endocannabinoid signalling in conditions of chronic stress (see (Gorzalka et al., 2008; Patel and Hillard, 2008) for reviews of this topic); however, this topic was beyond the scope of the current review.

Overall, the studies to date suggest that the interactions between the endocannabinoid system and the HPA axis are both complex and reciprocal. The general picture that emerges from these studies is that with respect to the neuroendocrine aspect of stress, endocannabinoid signalling appears to regulate both the activation and termination of the HPA axis in response to stress through a reduction in AEA and an increase in 2-AG, respectively. Accordingly, it is becoming increasingly apparent that the endocannabinoid system is an integral regulatory force on HPA axis activation and stress responsivity. This phenomenon likely contributes to the putative therapeutic role this system may play for mood and anxiety disorders which are often associated with heightened sensitivity to stress (Gorzalka et al., 2008; Hill et al., 2009a)

At the neurobehavioral level, a similar pattern emerges such that induction of endocannabinoid signalling (probably 2-AG) in response to stress appears to constrain neuronal activation and transmitter release within the corticolimbic stress circuit and consequentially modulate the expression of motivated behaviours. On the other hand, the stress-induced decline of AEA signaling appears to be involved in the development of heightened emotionality following stress exposure. Thus, similar to neuroendocrine function a duality emerges in the role of the endocannabinoid system following stress exposure with bidirectional regulation of endocannabinoid ligands contributing to both activational neurobehavioral responses to stress and the return to homeostasis thereafter.

Continuing research in this area is required to completely understand the exact nature of this system in stress responsivity and recovery, including both the exact neural circuits with which this signalling is integral and the mechanisms by which these changes in endocannabinoid signalling occur in response to both stress and glucocorticoids.

## References

- Aguado T, Monory K, Palazuelos J, Stella N, Cravatt B, Lutz B, Marsicano G, Kokaia Z, Guzman M, Galve-Roperh I. The endocannabinoid system drives neural progenitor proliferation. *FASEB J*. 2005; 19:1704–1706. [PubMed: 16037095]
- Ahn K, McKinney MK, Cravatt BF. Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. *Chem Rev*. 2008; 108:1687–1707. [PubMed: 18429637]
- 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]
- 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]
- Benito C, Tolon RM, Pazos MR, Nunez E, Castillo AI, Romero J. Cannabinoid CB2 receptors in human brain inflammation. *Br J Pharmacol*. 2008; 153:277–285. [PubMed: 17934510]
- Bisogno T. Endogenous cannabinoids: structure and metabolism. *J Neuroendocrinol*. 2008; 20 1:1–9. [PubMed: 18426492]
- Blednov YA, Cravatt BF, Boehm SL 2nd, Walker D, Harris RA. Role of endocannabinoids in alcohol consumption and intoxication: studies of mice lacking fatty acid amide hydrolase. *Neuropsychopharmacology*. 2007; 32:1570–1582. [PubMed: 17164820]
- Campolongo P, Roozendaal B, Trezza V, Hauer D, Schelling G, McGaugh JL, Cuomo V. Endocannabinoids in the rat basolateral amygdala enhance memory consolidation and enable glucocorticoid modulation of memory. *Proc Natl Acad Sci U S A*. 2009; 106:4888–4893. [PubMed: 19255436]
- Coddington E, Lewis C, Rose JD, Moore FL. Endocannabinoids mediate the effects of acute stress and corticosterone on sex behavior. *Endocrinology*. 2007; 148:493–500. [PubMed: 17095597]
- Cota D, Marsicano G, Tschop 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]
- Degroot A, Kofalvi A, Wade MR, Davis RJ, Rodrigues RJ, Rebola N, Cunha RA, Nomikos GG. CB1 receptor antagonism increases hippocampal acetylcholine release: site and mechanism of action. *Mol Pharmacol*. 2006; 70:1236–1245. [PubMed: 16855179]
- 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 S, Boudaba C, Popescu IR, Weng FJ, Harris C, Marcheselli VL, Bazan NG, Tasker JG. Activity-dependent release and actions of endocannabinoids in the rat hypothalamic supraoptic nucleus. *J Physiol*. 2005a; 569:751–760. [PubMed: 16239276]
- Di S, Malcher-Lopes R, Halmos KC, Tasker JG. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. *J Neurosci*. 2003; 23:4850–4857. [PubMed: 12832507]
- Di S, Malcher-Lopes R, Marcheselli VL, Bazan NG, Tasker JG. Rapid glucocorticoid-mediated endocannabinoid release and opposing regulation of glutamate and gamma-aminobutyric acid inputs to hypothalamic magnocellular neurons. *Endocrinology*. 2005b; 146:4292–4301. [PubMed: 15994343]

- Di S, Maxson MM, Franco A, Tasker JG. Glucocorticoids regulate glutamate and GABA synapse-specific retrograde transmission via divergent nongenomic signaling pathways. *J Neurosci*. 2009; 29:393–401. [PubMed: 19144839]
- 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]
- Evanson NK, Ulrich-Lai YM, Furay AR, Tasker JG, Herman JP. Hypothalamic paraventricular cannabinoid receptor signaling in fast feedback inhibition of the hypothalamus-pituitary-adrenal axis response to acute restraint stress. *Society for Neuroscience Abstracts*. 2007; 197:195.
- Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev*. 2003; 83:1017–1066. [PubMed: 12843414]
- Gorzalka BB, Hill MN, Chang SC. Male-female differences in the effects of cannabinoids on sexual behavior and gonadal hormone function. *Horm Behav*. 2009
- 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, Barna I, Barsvari B, Gyimesi Pelczer K, Yasar S, Panlilio LV, Goldberg S. Interactions between environmental aversiveness and the anxiolytic effects of enhanced cannabinoid signaling by FAAH inhibition in rats. *Psychopharmacology (Berl)*. 2009
- 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]
- Hansson AC, Bermudez-Silva FJ, Malinen H, Hyytia P, Sanchez-Vera I, Rimondini R, Rodriguez de Fonseca F, Kunos G, Sommer WH, Heilig M. Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. *Neuropsychopharmacology*. 2007; 32:117–126. [PubMed: 16482090]
- 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, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, Cullinan WE. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol*. 2003; 24:151–180. [PubMed: 14596810]
- Hill MN, Hillard CJ, Bambico FR, Patel S, Gorzalka BB, Gobbi G. The therapeutic potential of the endocannabinoid system for the development of a novel class of antidepressants. *Trends Pharmacol Sci*. 2009a; 30:484–493. [PubMed: 19732971]
- Hill MN, Kambo JS, Sun JC, Gorzalka BB, Galea LA. Endocannabinoids modulate stress-induced suppression of hippocampal cell proliferation and activation of defensive behaviours. *Eur J Neurosci*. 2006; 24:1845–1849. [PubMed: 17067290]
- Hill MN, Karatsoreos IN, Hillard CJ, McEwen BS. Rapid induction of limbic endocannabinoid signaling in vivo by glucocorticoid hormones. *Psychoneuroendocrinology*. 2009b Under review.
- 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*. 2009c in press.
- Hill, MN.; McLaughlin, RJ.; Viau, V.; Gorzalka, BB.; Hillard, CJ. Stress-induced alterations in limbic endocannabinoid content in the rat: Correlations to HPA axis activation. 17th Annual Symposium of the International Cannabinoid Research Society; 2007. p. 23
- 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*. 2009d; 34:1257–1262. [PubMed: 19394765]
- Hillard CJ. Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. *Prostaglandins Other Lipid Mediat*. 2000; 61:3–18. [PubMed: 10785538]
- Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R, Krey JF, Walker JM, Holmes PV, Crystal JD, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D. An endocannabinoid mechanism for stress-induced analgesia. *Nature*. 2005; 435:1108–1112. [PubMed: 15973410]

- Howlett AC. The cannabinoid receptors. *Prostaglandins Other Lipid Mediat.* 2002; 68-69:619–631. [PubMed: 12432948]
- 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]
- Keller-Wood ME, Dallman MF. Corticosteroid inhibition of ACTH secretion. *Endocr Rev.* 1984; 5:1–24. [PubMed: 6323158]
- Laugero KD, Bell ME, Bhatnagar S, Soriano L, Dallman MF. Sucrose ingestion normalizes central expression of corticotropin-releasing-factor messenger ribonucleic acid and energy balance in adrenalectomized rats: a glucocorticoid-metabolic-brain axis? *Endocrinology.* 2001; 142:2796–2804. [PubMed: 11415998]
- 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]
- 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]
- McGregor IS, Hargreaves GA, Apfelbach R, Hunt GE. Neural correlates of cat odor-induced anxiety in rats: region-specific effects of the benzodiazepine midazolam. *J Neurosci.* 2004; 24:4134–4144. [PubMed: 15115808]
- Mikics E, Barsy B, Barsvari B, Haller J. Behavioral specificity of non-genomic glucocorticoid effects in rats: effects on risk assessment in the elevated plus-maze and the open-field. *Horm Behav.* 2005; 48:152–162. [PubMed: 16042965]
- Mikics E, Kruk MR, Haller J. Genomic and non-genomic effects of glucocorticoids on aggressive behavior in male rats. *Psychoneuroendocrinology.* 2004; 29:618–635. [PubMed: 15041085]
- Moldrich G, Wenger T. Localization of the CB1 cannabinoid receptor in the rat brain. An immunohistochemical study *Peptides.* 2000; 21:1735–1742.
- Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993; 365:61–65. [PubMed: 7689702]
- Naidu PS, Varvel SA, Ahn K, Cravatt BF, Martin BR, Lichtman AH. Evaluation of fatty acid amide hydrolase inhibition in murine models of emotionality. *Psychopharmacology (Berl).* 2007; 192:61–70. [PubMed: 17279376]
- Ohno-Shosaku T, Maejima T, Kano M. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron.* 2001; 29:729–738. [PubMed: 11301031]
- 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]
- Orchinik M, Murray TF, Franklin PH, Moore FL. Guanyl nucleotides modulate binding to steroid receptors in neuronal membranes. *Proc Natl Acad Sci U S A.* 1992; 89:3830–3834. [PubMed: 1570300]
- Orchinik M, Murray TF, Moore FL. A corticosteroid receptor in neuronal membranes. *Science.* 1991; 252:1848–1851. [PubMed: 2063198]
- Palazuelos J, Aguado T, Egia A, Mechoulam R, Guzman M, Galve-Roperh I. Non-psychoactive CB2 cannabinoid agonists stimulate neural progenitor proliferation. *FASEB J.* 2006; 20:2405–2407. [PubMed: 17015409]
- Parolaro D. Presence and functional regulation of cannabinoid receptors in immune cells. *Life Sci.* 1999; 65:637–644. [PubMed: 10462064]
- Patel S, Hillard CJ. Adaptations in endocannabinoid signaling in response to repeated homotypic stress: a novel mechanism for stress habituation. *Eur J Neurosci.* 2008; 27:2821–2829. [PubMed: 18588527]
- 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. [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*. 2005; 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]
- Racz I, Bilkei-Gorzo A, Toth ZE, Michel K, Palkovits M, Zimmer A. A critical role for the cannabinoid CB1 receptors in alcohol dependence and stress-stimulated ethanol drinking. *J Neurosci*. 2003; 23:2453–2458. [PubMed: 12657705]
- Rademacher DJ, Meier SE, Shi L, Ho WS, Jarrhian 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]
- Roosendaal B, McEwen BS, Chattarji S. Stress, memory and the amygdala. *Nat Rev Neurosci*. 2009; 10:423–433. [PubMed: 19469026]
- Rose JD, Moore FL. A neurobehavioral model for rapid actions of corticosterone on sensorimotor integration. *Steroids*. 1999; 64:92–99. [PubMed: 10323677]
- Schlicker E, Kathmann M. Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci*. 2001; 22:565–572. [PubMed: 11698100]
- 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]
- 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]
- 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]
- Sugiura T, Waku K. Cannabinoid receptors and their endogenous ligands. *J Biochem*. 2002; 132:7–12. [PubMed: 12097154]
- Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience*. 1998; 83:393–411. [PubMed: 9460749]
- 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
- 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]
- Wilson RI, Kunos G, Nicoll RA. Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron*. 2001; 31:453–462. [PubMed: 11516401]

**Table 1**

The effects of acute stress on the tissue content of the endocannabinoid ligands anandamide (AEA) and 2-arachidonoylglycerol (2-AG) in discrete brain regions.

Species/Strain	Stressor	Brain Region	AEA	2-AG	Reference
ICR mice	30 min restraint stress	Hypothalamus	/	-	Patel et al., 2004
ICR mice	30 min restraint stress	Forebrain	/	/	Patel et al., 2005
		Amygdala	-	/	
		Cerebellum	/	/	
ICR mice	30 min restraint stress	Prefrontal Cortex	/	/	Rademacher et al., 2008
		Amygdala	-	/	
		Ventral Striatum	/	/	
Sprague-Dawley rats	30 min restraint stress	Prefrontal Cortex	-	+	Hill et al., 2007
		Hippocampus	-	+	
		Hypothalamus	/	+	
Sprague-Dawley rats	30 min restraint stress	Amygdala	-	/	Hill et al., 2009c
Sprague-Dawley rats	3 min footshock	Periaqueductal			
		Grey (PAG)	+	+	Hohmann et al., 2005
		Occipital Cortex	/	/	

**Footnote:** / = no change; - = significant reduction; + = significant increase