Adaptations in Endocannabinoid Signaling in Response to Repeated Homotypic Stress: A Novel Mechanism for Stress Habituation

Sachin Patel[1] and **Cecilia J. Hillard**[2]

[1]*Department of Psychiatry, Vanderbilt University Medical Center, Nashville, TN 37212*

[2]*Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226*

Abstract

Daily life stressors are a major environmental factor contributing to precipitation and exacerbation of mental illness. Animal models using repeated homotypic stress induce anxious and depressive phenotypes and are used to study the pathophysiology of affective disorders. Here we discuss data demonstrating that repeated homotypic stress produces temporally and anatomically distinct changes in endocannabinoid signaling components within stress-responsive brain regions. We also present evidence describing the neural and behavioral correlates of these adaptations in endocannabinoid signaling. These data support a role for endocannabinoid signaling in the CNS response to chronic, homotypic stress; specifically in the process of stress-response habituation. The clinical implications of these findings for the pathophysiology and treatment of affective disorders are discussed.

Keywords

restraint; anandamide; 2-arachidonoylglycerol; depression; anxiety; marijuana; CB1 cannabinoid receptor; mouse; *N*-arachidonylethanolamine; fatty acid amide hydrolase; rimonabant

INTRODUCTION

One of the environmental factors that consistently precipitates and exacerbates mental illnesses, including depression and anxiety, is repeated life stress (Dinan, 2005). Importantly, homotypic stressors that occur on a daily basis, such as marital problems, medical problems, work stress, and poverty, are associated with increased depressive symptoms (Caspi *et al.*, 2003; Hammen *et al.*, 2004; Hammen, 2005; Southwick *et al.*, 2005; Keller *et al.*, 2007; Robertson Blackmore *et al.*, 2007). The development of animal models that recapitulate anxious and depressive symptoms, and associated physiological changes, has allowed for experimental investigations into the contributions of stress to mental illness and the mechanisms underlying stress-adaptation (Pittenger & Duman, 2007). One of these experimental models is repeated restraint stress. Repeated application (between 5 and 21 consecutive days, from 30 minutes to 8 hours daily depending on the paradigm) of physical restraint to rodents has been shown to induce depressive and anxious phenotypes (Vyas & Chattarji, 2004; Mitra *et al.*, 2005; Kim & Han, 2006), immunosuppression (Sheridan *et al.*,

Correspondence to either: Sachin Patel, MD, PhD, Department of Psychiatry, Vanderbilt University School of Medicine, Vanderbilt Psychiatric Hospital, Suite 313, 1601 23rd Ave South, Nashville TN, 37212, sachin.patel@vanderbilt.edu, Tel: (615) 327-7080, FAX: (615) 322-1901 or Cecilia J. Hillard Ph.D., Department of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53216, Tel: 414 456 8493, Fax: 414 456 6545, Email: chillard@mcw.edu.

1994; Raison & Miller, 2001; Shi *et al.*, 2003) and cognitive impairments (Radecki *et al.*, 2005; Trofimiuk *et al.*, 2005; Walesiuk *et al.*, 2005; Lupien *et al.*, 2007; Wright & Conrad, 2007); symptoms often experienced in depressive illness (Mathews & MacLeod, 2005; Schneiderman *et al.*, 2005; Irwin & Miller, 2007). Repeated restraint also increases plasma glucocorticoid concentrations, causes adrenal gland hypertrophy, and reduces body weight (Melia *et al.*, 1994; Kim & Han, 2006).

Behavioral and hormonal responses to repeated and predictable exposure to homotypic stressors (such as restraint) exhibit habituation. Habituation is a progressive decrease in the expression of stress responses after repeated applications of the same stressor. Habituation is stressor specific (Kant *et al.*, 1985; Lachuer *et al.*, 1994; Melia *et al.*, 1994), dependent upon the inter-stimulus duration (De Boer *et al.*, 1990), and initial stressor intensity (Natelson *et al.*, 1988). Much of the data regarding the mechanisms of stress habituation have focused on the role of glucocorticoids (Kant *et al.*, 1985; Melia *et al.*, 1994; Cole *et al.*, 2000; Jaferi *et al.*, 2003; Jaferi & Bhatnagar, 2006). However, recent studies have also implicated the endocannabinoid signaling system in this process (Patel *et al.*, 2005b; Kamprath *et al.*, 2006).

ENDOCANNABINOID SIGNALING

The endocannabinoids, *N*-arachidonylethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG), are neuroactive lipids that are produced within the brain by neurons and glial cells and function primarily as interneuronal signaling molecules (Freund *et al.*, 2003; Piomelli, 2003; Bisogno *et al.*, 2005). The endocannabinoids are not stored or released vesicularly, but are synthesized and/or released in response to altered neuronal activity. The syntheses of AEA and 2-AG occur via separate enzymatic cascades and are evoked by neuronal depolarization, elevations in intracellular calcium, and activation of several metabotropic and excitatory ionotropic neurotransmitter receptors (DiMarzo *et al.*, 1994; Sugiura *et al.*, 2002; Freund *et al.*, 2003; Jung *et al.*, 2005; Liu *et al.*, 2006). The predominant endocannabinoid receptor found within the central nervous system is the G-protein coupled receptor, CB1, whose subcellular localization is primarily axonal terminals (Piomelli, 2003; Chevaleyre *et al.*, 2006)). CB1 receptors are present at high density within limbic brain structures including the amygdala, hippocampus, and prefrontal cortex (Tsou *et al.*, 1998; Egertova *et al.*, 2003; Katona *et al.*, 2006). Activation of CB1 receptors on axon terminals results in inhibition of neurotransmitter release via $G_{i/o}$-coupled intracellular signaling pathways (Chevaleyre *et al.*, 2006). Considerable evidence supports the hypothesis that endocannabinoids function as activity-dependent, retrograde inhibitors of neurotransmitter release. In particular, endocannabinoid/CB1 signaling subserves several forms of short and long-term neuronal plasticity, including depolarization-induced suppression, and long-term depression of excitatory and inhibitory neurotransmission within stress responsive brain regions including the prefrontal cortex (PFC) (Auclair *et al.*, 2000; Leforcade *et al.*, 2007), amygdala (Marsicano *et al.*, 2002; Azad *et al.*, 2004), and nucleus accumbens (Robbe *et al.*, 2002; Mato *et al.*, 2007). 2-AG is primarily, but not exclusively, degraded by monoacylglycerol lipase (MGL), which is located within presynaptic terminals (Dinh *et al.*, 2002). In contrast, AEA is degraded by an intracellular serine hydrolase, fatty acid amide hydrolase (FAAH), that is found predominantly in neurons postsynaptic to axon terminals expressing the CB1 receptor (Tsou *et al.*, 1998b).

Analysis of brain regional concentrations of the endocannabinoids by mass spectrometry has led the observation that they exhibit dynamic and often divergent changes in response to a variety of behavioral and pharmacological stimuli. Recent studies have utilized this technique, in combination with behavioral and physiological studies, to explore the effects of repeated, homotypic stress on the endocannabinoid system. We summarize these findings below and discuss their relevance to the pathophysiology and treatment of affective disorders.

REPEATED EXPOSURE TO RESTRAINT STRESS INDUCES CHANGES IN ENDOCANNABINOID SIGNALING COMPONENTS

We have examined the effects of repeated restraint stress, 30 minutes daily for varying durations, on endocannabinoid contents in different brain regions of mice (Patel *et al.*, 2004; Patel *et al.*, 2005b; Rademacher *et al.*, 2008). Several consistent patterns have been observed for both 2-AG and AEA in response to restraint stress. These data indicate temporally dynamic and regionally divergent (cortical vs. striatal) effects of repeated restraint stress on these two endocannabinoid ligands (see Figure 1). A single application of 30 minute restraint does not alter the content of 2-AG within the limbic forebrain, medial prefrontal cortex (mPFC), ventral striatum, hippocampus*, amygdala, or cerebellum ((Patel *et al.*, 2005b) and *Patel and Hillard, unpublished data), but decreases 2-AG content within the hypothalamus (Patel *et al.* 2004). In contrast, increasing the number of consecutive daily restraint episodes to 5, 7 and 10 results in a progressive increase in 2-AG content within the mPFC, limbic forebrain, amygdala, hippocampus, and hypothalamus immediately after the last restraint episode (Patel *et al.*, 2005b, Rademacher *et al.*, 2008). Interestingly, chronic treatment of rats with corticosterone also results in increased 2-AG content in the amygdala (Hill *et al.* 2005), suggesting that the effect of repeated restraint on amygdalar 2-AG content could be secondary to a persistent increase in glucocorticoid signaling. In contrast to these brain regions, a significant decrease in ventral striatal 2-AG content was seen after the 7th restraint (Rademacher *et al.*, 2008).

Taken together, these data indicate that multiple exposures to the same stressor, in this case restraint, recruits or activates 2-AG-mediated signaling within cortical-like brain regions (mPFC, amygdala, hippocampus) and the hypothalamus. This effect can be considered a form of sensitization since a single episode of restraint did not increase 2-AG contents in these regions. Sensitization of the 2-AG response could occur via several possible mechanisms: first, 2-AG degradation could be reduced; second, an increase in the release of a neurotransmitter (such as glutamate) in response to stress could activate 2-AG synthetic enzymes phospholipase C (PLC) and/or diacylglycerol lipase (DAGL); or third, repeated stress exposure could up-regulate the 2-AG synthetic enzymes. With regard to the first possibility, 2-acylglycerol hydrolysis is not decreased ex vivo in cytosolic preparations from the amygdala, ventral striatum or mPFC of mice exposed to 10 episodes of restraint (Rademacher *et al.*, 2008). With regard to the second possibility, glutamate efflux within the PFC is robust during the first exposure to a stressor, but shows habituation such that by the third exposure glutamate efflux is significantly reduced compared to the first exposure (see Moghaddam 2002). This suggests that increase in 2-AG content observed after repeated restraint stress occurs in the face of decreasing stimulatory drive (i.e. glutamate release), and is thus likely mediated by the third possibility, an increase in 2-AG synthetic capacity. While Dwivedi and colleagues reported that PLCß expression is decreased after repeated stress in the frontal cortex (Dwivedi *et al.*, 2005), the effects of repeated restraint on the activity and expression of both other PLC isoforms and DAGL have not been studied. Interestingly, these data suggest the habituated glutamate response to repeated stress within the PFC could be mediated by increased 2-AG signaling, since 2-AG can decrease presynaptic glutamate release within the PFC ((Auclair *et al.*, 2000;Lafourcade *et al*. 2007) and see below). In other words, the directionality of the interaction between 2-AG and glutamate could be 2-AG affecting glutamate release rather than glutamate release acting as the primary driving force for 2-AG release under these conditions.

AEA contents are also affected by stress, but the pattern is different from that seen with 2-AG. In the mPFC and amygdala, restraint stress decreases AEA content regardless of the number of prior exposures (Patel *et al.* 2005b, Rademacher *et al*. 2008). In fact, in both regions, the reduction in AEA tends to become more pronounced as the number of restraint episodes increases, although this has not been a consistent finding. FAAH is up-regulated in the amygdala and mPFC (as measured by an increase in its Vmax) after 10 episodes of restraint.

This change is consistent with a greater effect of stress on AEA after multiple exposures but does not explain the effect of the first restraint. It is our current hypothesis that AEA synthesis is tonically "on" in these brain regions when the mice are in a calm, normal state. The application of stress results in a reduction of AEA synthesis through a mechanism that does not habituate or sensitize to a significant degree. Alternatively, it is possible that acute restraint results in the rapid release and subsequent degradation of a limited supply of preformed AEA, which would be measured as a decrease in tissue content if stores of AEA have not had time to be replenished. This hypothesis suggests a limited capacity for "on-demand synthesis", and could be tested by analyzing AEA precursor content together with the activity of AEA biosynthetic enzymes in response to acute restraint stress.

Interestingly, the contents of AEA and two other *N*-acylethanolamines (palmitoylethanolamide and oleoylethanolamide) are significantly increased in the ventral striatum after 10 episodes of restraint. These changes are accompanied by a decrease in the Vmax for FAAH, which suggests that the increased content within this region is due to decreased catabolism of this family of lipids (Rademacher *et al.* 2008).

We have also determined whether repeated restraint stress alters CB1 receptor density or affinity for the agonist, CP55940. Neither the Kd nor Bmax for CP55940 binding to the CB1 receptor were altered by acute or repeated restraint stress within any brain region examined (Rademacher *et al.*, 2008). These data suggest that the major changes in the endocannabinoid system in response to repeated restraint stress occur at the level of the endogenous ligands AEA and 2-AG rather than at the level of their cognate receptor (see Figure 1), however changes in signaling efficacy through the CB1 receptor remain an open possibility.

Several important questions remain to be answered regarding the endocannabinoid response to repeated restraint stress. First, are the long-term changes in AEA and/or 2-AG dependent upon application of the last restraint *per se*, or are they adaptive changes that are dependent upon previous restraint stress exposure only? Examination of AEA and 2-AG tissue contents a longer time after the termination of the last restraint episode would answer this question. Second, are these sensitized adaptations in 2-AG signaling specific to the stressor (i.e. restraint), or do they generalize to any subsequent aversive experience? Determining if the sensitized 2-AG response is stressor specific would strengthen the argument for a role in habituation, since habituation is stressor specific (i.e. requires repeated exposure to the *same* stressor). Examination of 2-AG content after application of a novel stressor subsequent to the repeated restraint protocol would differentiate between these two possibilities. Third, do the changes in tissue endocannabinoids reflect changes in the endocannabinoids that are available to activate the CB1 receptor? This is an important issue and caveat of the data presented thus far. Recent data from Parsons and colleagues (Caille *et al*. 2007) suggest that dialyzable 2-AG content is a fraction of the content measured in tissue pieces. Therefore, it will be important to confirm the changes in endocannabinoid content using techniques, such as microdialysis, that can sample a pool that is more likely to be involved in signaling. Although these and other questions remain, our data support the hypothesis that robust and reproducible changes in endocannabinoid content occur in response to repeated, homotypic stress and suggest a prominent role for this signaling system in the adaptive responses to stress, some of which are described below.

ENDOCANNABINOID SIGNALING MODULATES LIMBIC NEURONAL RESPONSES TO REPEATED STRESS

Since the endocannabinoid 2-AG has been implicated in the inhibition of glutamate release within the PFC and other brain regions (Lafourcade *et al*. 2007), and repeated restraint stress increases 2-AG content within limbic brain structures, we explored the role of stress-induced

activation of endocannabinoid signaling on neuronal activation using Fos expression as a marker of neuronal excitation (Patel *et al.*, 2005b). Consistent with previous data, a single episode of restraint stress increased Fos expression within limbic structures including the mPFC, lateral septum, and the medial amygdala. All of these responses were significantly reduced during the 5th restraint episode, a time at which limbic forebrain 2-AG content was increased. This lead us to test the hypothesis that the habituation of neuronal activation by stress in these regions was due to an increase in 2-AG content. To test this hypothesis, we administered the CB1 receptor antagonist, rimonabant, prior to the 5th restraint application to block the effects of 2-AG on CB1 receptor signaling. Administration of rimonabant prior to the 5th restraint episode blocked the habituation and returned Fos expression to levels observed during the first restraint application in the mPFC and lateral septum (Patel *et al.*, 2005b). We interpret these data to indicate that the progressive increase in 2-AG observed after repeated restraint serves to dampen neuronal activation induced by restraint in these brain regions and thereby contributes to the habituation of limbic neuronal responses that occurs in response to repeated homotypic stress application.

A synaptic mechanism subserving this phenomenon can be hypothesized based on recent data from Manzoni and colleagues who demonstrated that CB1 receptors are located on excitatory presynaptic terminals in the mPFC and the 2-AG synthetic enzyme, DAGL, is located within dendrites of PFC pyramidal neurons (Lafourcade *et al.*, 2007). Furthermore, activation of CB1 receptors results in a decrease in excitatory transmission onto PFC pyramidal neurons (Lafourcade *et al.*, 2007). More interestingly, tetanic stimulation-induced long-term depression of excitatory transmission is dependent upon CB1 receptor activation by 2-AG (Lafourcade *et al.*, 2007). Based on these data, we suggest the following model for the role of 2-AG in the habituation of neuronal activation in response to repeated restraint stress (Figure 2). Acute restraint stress activates glutamatergic inputs to the mPFC which initiates both the release of 2-AG and Fos protein expression. 2-AG, in turn, activates CB1 receptors on the glutamatergic terminals resulting in decreased glutamate release; however, this does not occur robustly since rimonabant had no effect on Fos expression following a single restraint in these regions. Upon exposure to the 5th restraint, stress-related afferent input patterns to the mPFC result in synthesis of larger amounts of 2-AG (possibly via an activity-dependent, up-regulation of postsynaptic 2-AG synthetic enzymes), resulting in a strong reduction in glutamate release and downstream Fos expression. The net result is a habituated Fos response to repeated restraint, and is consistent with the habituated PFC glutamate efflux observed by microdialysis after repeated stress exposure (Moghaddam 2002). However, when the CB1 receptor antagonist is administered prior to the 5th restraint, the 2-AG-CB1 receptor-dependent synaptic suppression is blocked, resulting in loss of habituation. Determining whether CB1 receptor antagonist administration prior to the 5th restraint episode also reverses the habituated glutamate efflux would further validate this model.

ENDOCANNABINOID SIGNALING MODULATES BEHAVIORAL RESPONSES TO REPEATED STRESS

When restrained in plastic conical tubes, mice initially exhibit escape behaviors including biting, turning and chewing. These behaviors subside during the course of the 30 minute restraint episode, and show significant habituation during the 5th restraint episode (Patel *et al.*, 2005b). During the 5th restraint episode, when 2-AG is elevated in limbic brain regions, administration of rimonabant re-instates the escape behaviors, although not to the extent that was observed during the first restraint episode (Patel *et al.*, 2005b). These data suggest a partial role for 2-AG signaling in behavioral habituation during repeated restraint exposure. Similarly, Kamprath *et al.* (2006) have provided evidence that the CB1 receptor is required for the habituation of innate fear behaviors in mice. In this paradigm, presentation of the tone stimulus used in fear conditioning paradigms (preceded by a sensitizing shock) results in freezing

behavior that habituates following repeated presentations; this represents a non-associative component of extinction conditioned fear behavior (Kamprath & Wotjak, 2004). Mice lacking CB1 receptors do not show habituation of these innate fear responses after repeated tone presentation (Kamprath *et al.*, 2006). These authors suggest that the impairments in extinction of conditioned fear behavior observed in CB1 null mice, and after CB1 receptor blockade, are a result of an impaired "habituation component" of the extinction process (Kamprath *et al.*, 2006). Taken together, these data support the hypothesis that the endocannabinoid system is involved in behavioral habituation to repeated, homotypic stress exposure.

We have also explored the role of the endocannabinoid system in the anhedonia induced by repeated stress exposure (Rademacher & Hillard, 2007). Anhedonia, a prominent symptom of depression in humans, is seen as a reduction in sucrose preference in mice exposed to repeated stress. This reduction in sucrose preference is present between the 1st and 7th restraint episodes and was reversed by pretreatment of the mice with inhibitors of FAAH and enhanced by blockade of the CB1 receptor. These findings are consistent with a stress-buffering role for endocannabinoid signaling. However, these data also suggest a growing reliance on endocannabinoid signaling as the number of restraint exposures increases since administration of rimonabant prior to the 10th restraint episode severely reduced sucrose preference (Rademacher & Hillard, 2007). Therefore, although the repeated exposure to restraint did not habituate with regard to its effect on sucrose consumption, the endocannabinoid system was nonetheless progressively recruited and acted to reverse the effects of the stress on reward-motivated behavior. These data, together with the increase in cortical 2-AG content and ventral striatal AEA content observed during repeated restraint exposure, support the hypothesis that one or both of these signaling systems functions as a neuroadaptive mechanism to counteract the anhedonic phenotype induced by repeated restraint stress. This hypothesis is further supported by our finding that direct and indirect CB1 agonists reverse restraint stress-induced decreases in sucrose preference prior to the 10th restraint exposure (Rademacher & Hillard, 2007), and the data of others that low levels of CB1 receptor activation facilitate operant responding for food and drugs of abuse in non-stressed animals (Higgs *et al.*, 2005; Solinas & Goldberg, 2005; Solinas *et al.*, 2005). Taken together, these data support the hypothesis that the endocannabinoid system facilitates motivated behavior in a generalized fashion and thereby counteracts stress-induced anhedonia.

ENDOCANNABINOID SIGNALING MODULATES NEUROENDOCRINE RESPONSES TO REPEATED STRESS

Endocannabinoid signaling also plays a role in adaptation of the hypothalamic-pituitary-adrenal (HPA) axis to repeated restraint stress. Repeated activation of the HPA axis by restraint stress also demonstrates habituation as measured by a progressive decrease in plasma corticosterone with increasing numbers of restraint episodes (Patel *et al.*, 2004). As mentioned above, 2-AG is decreased in the hypothalamus after the first restraint exposure, while AEA is unchanged. However, 2-AG is increased after the 5th restraint application, at which time the corticosterone response shows habituation (Patel *et al.*, 2004). This finding, combined with the data that low doses of direct CB1 agonists and indirect CB1 agonists can inhibit stress-induced corticosterone release, lead us to hypothesize that 2-AG synthesis is increased upon repeated restraint application and that this change contributes to habituated HPA-axis response to repeated restraint stress (Patel *et al.*, 2004).

CONCLUSIONS AND CLINICAL IMPLICATIONS

The data reviewed above suggests a role for endocannabinoid signaling in the adaptation to repeated homotypic stress. Alterations in endocannabinoid signaling in response to repeated homotypic stress appear to be at the level of endogenous ligands rather than changes in CB1

receptor density or agonist affinity, although it is possible that changes in signal transduction efficacy have occurred. Alterations in endocannabinoid content appear to be selective for stress-sensitive brain regions since there were no changes observed within the cerebellum, a brain region mainly involved in motor coordination in rodents (although the cerebellum has been implicated in cognitive functioning in humans (see (Thach, 2007)). Within brain regions that exhibited changes in AEA and 2-AG content with repeated restraint stress, two general patterns were observed. A cortical-like pattern was observed within the mPFC, amygdala, and hippocampus (Figure 2). Although not a cortical structure, the hypothalamus showed a similar pattern, suggesting similar mechanisms could regulate endocannabinoid signaling in all of these regions. A different pattern was observed within the ventral striatum. These differential patterns could have implications for the pathophysiology and treatment of stress-related psychiatric disorders including depression, anxiety and post-traumatic stress disorder (PTSD).

In the brain regions exhibiting the cortical pattern of endocannabinoid changes, long-term decreases in tissue content of AEA are likely mediated by increased degradation. These data suggest that repeated stress induces an AEA deficiency in these cortical regions and that pharmacological augmentation or normalization of AEA signaling could represent a promising approach to the treatment of anxiety and depression. In support of this hypothesis, FAAH inhibitors (that increase brain AEA levels) have anti-anxiety and anti-depressant properties (Kathuria *et al.*, 2003; Gobbi *et al.*, 2005; Patel & Hillard, 2006; Bortolato *et al.*, 2007; Naidu *et al.*, 2007), and viral over-expression of FAAH within the PFC decreases AEA levels and induces anxiety behaviors in rats (Rubino *et al.*, 2007). In addition, we have found that women suffering from minor depression have decreased plasma AEA levels (Hill *et al.*, 2007), however another study did not detect changes in AEA content in the cerebrospinal fluid from patients with affective disorders compared to controls (Giuffrida *et al.*, 2004).

In contrast to AEA, 2-AG increases in cortical-like structures, and exhibits a sensitized and robust response to repeated restraint stress. We suggest this progressive increase contributes to the habituation of neuronal, endocrine, and behavioral responses to stress. The contribution of 2-AG-CB1 signaling to the expression of habituation, which is an important contributor to the extinction of conditioned fear responses, suggests that pharmacological augmentation of 2-AG signaling could have therapeutic potential for treatment of anxiety disorders including PTSD (Marsicano *et al.*, 2002; Suzuki *et al.*, 2004; Chhatwal *et al.*, 2005; Kamprath *et al.*, 2006).

Within subcortical regions such as the ventral striatum, our data indicate that repeated restraint results in a 2-AG deficit together with enhanced AEA signaling. Ventral striatal AEA signaling increases the hedonic impact of rewarding stimuli (Mahler *et al.*, 2007), increases mesolimbic dopamine activity (Solinas *et al.*, 2006), and likely counteracts stress-induced decreases in sucrose preference (Rademacher & Hillard, 2007). Together with observations that injections of 2-AG or compounds that increase 2-AG levels into the ventral striatum increase feeding behavior (Kirkham *et al.*, 2002; Soria-Gomez *et al.*, 2007), these data suggest that pharmacological augmentation of either AEA or 2-AG signaling could be used to treat anhedonic symptoms of depression.

The above hypotheses regarding the potential utility of indirect cannabinoid agonists in the treatment of affective disorders is consistent with the epidemiological data that the most common reasons given for continued cannabis use include the desire for its anxiolytic and antidepressant effects. However, paradoxical anxiety and panic are well known side effects of cannabis intoxication, especially at high doses and in stressful environmental contexts (Gregg *et al.*, 1976; Naliboff *et al.*, 1976; Szuster *et al.*, 1988; Thomas, 1996; Hall & Solowij, 1998; Reilly *et al.*, 1998). In this regard, we have shown that, in contrast to direct CB1 receptor agonists, a FAAH inhibitor does not have biphasic dose dependent effects, and does not

synergize with stress to active brain structures involved in the generation of anxiety, an effect that does occur with direct agonists (Patel *et al.*, 2004; Patel *et al.*, 2005a; Patel & Hillard, 2006). In summary, these data suggest the endocannabinoid system is an important mediator of stress habituation, and pharmacological augmentation of endocannabinoid signaling represents a mechanistically-sound target for the development of pharmacological agents for the treatment of affective disorders that could be devoid of the adverse side-effects of direct CB1 agonists.

Acknowledgements

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Abbreviations

AEA

N-arachidonylethanolamine

FAAH

fatty acid amide hydrolase

HPA

hypothalamic-pituitary-adrenal

PFC

prefrontal cortex

mPFC

medial prefrontal cortex

MGL

monoacylglycerol lipase

PTSD

post-traumatic stress disorder

CB1

type-1 cannabinoid receptor

2-AG

2-arachidonoylglycerol

PLC

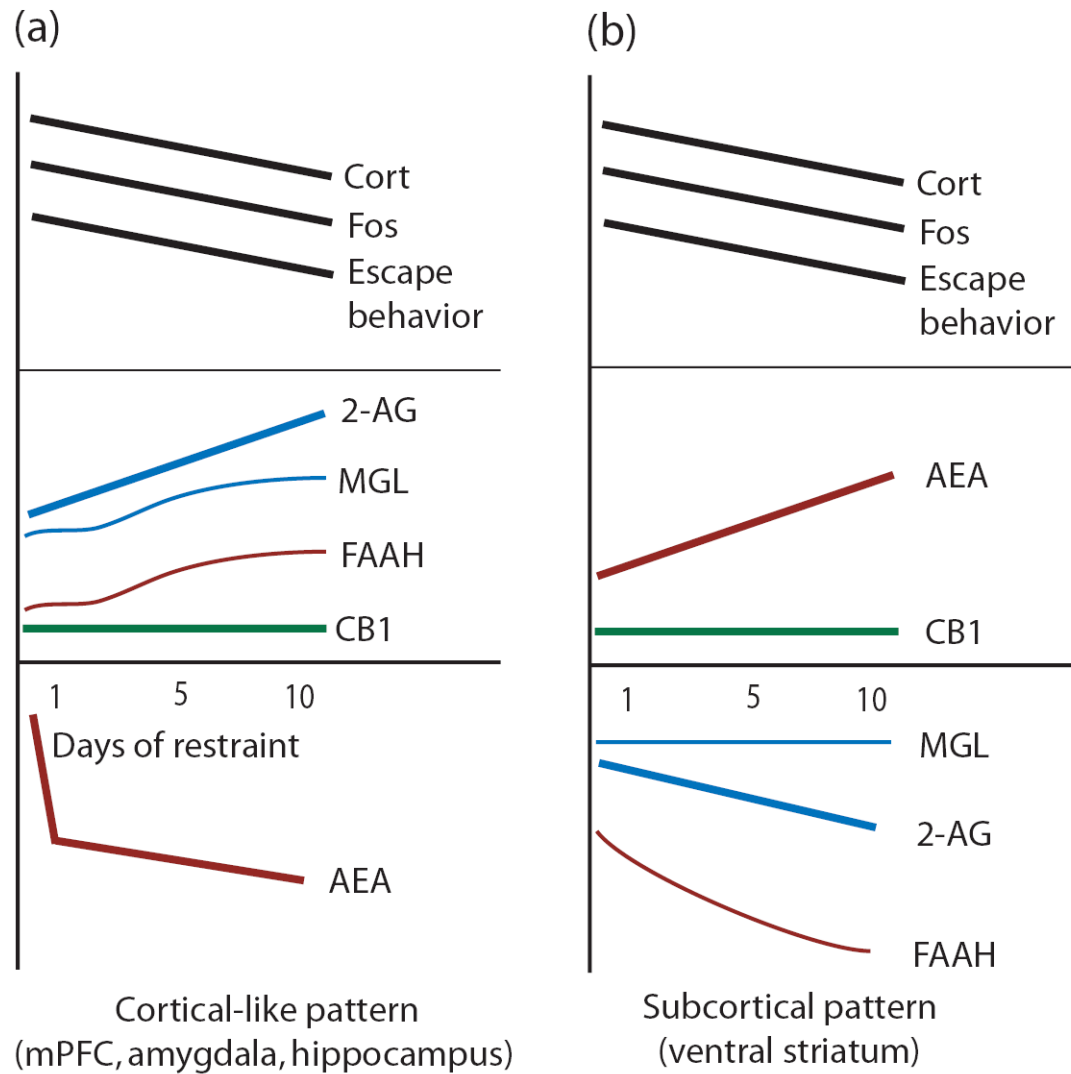   phospholipase C

DAGL

    diacylglycerol lipase

Figure 1.
Diagramatic representation of adaptations in endocannabinoid signaling components in response to repeated restraint stress. Top panels (black) represent the habituation of neuroendocrine, neuronal and behavioral habituation in response to repeated restraint. Bottom panels show the differential patterns of changes in endocannabinoid signaling components, AEA and 2-AG levels, CB1 receptor density, FAAH and MGL activity. The cortical pattern is shown in (a), whereas the sub-cortical pattern is shown in (b).
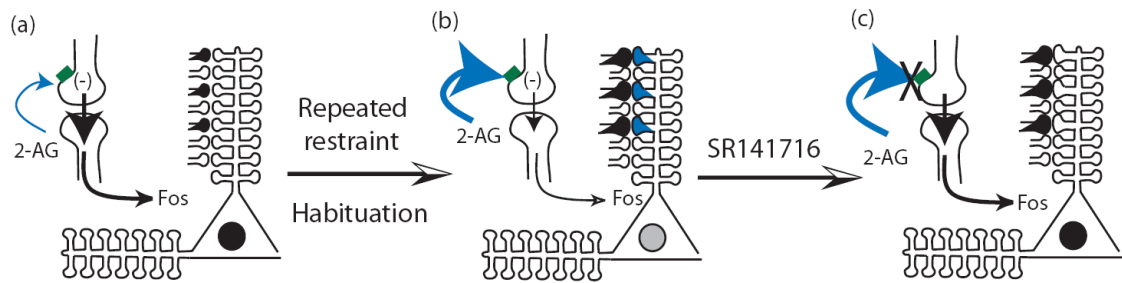
Figure 2.
Proposed mechanism by which 2-AG signaling, activated by repeated restraint stress, could contribute to the habituated neuronal responses. Initial exposure to stress (a) increases Fos expression (black nuclei) in limbic brain regions via activation of specific afferent synaptic activity patterns (black presynaptic terminals), this activation causes a small increases 2-AG signaling that serves to decrease presynaptic glutamatergic transmission onto mPFC pyramidal neurons via activation of CB1 receptors (green). After repeated stress application (b), an activity dependent up-regulation of 2-AG synthetic enzymes (blue post-synaptic dendritic spines) results in a large increase in 2-AG synthesis in response to stress. The large increase in 2-AG activates presynaptic CB1 receptors which decreases presynaptic glutamate release, post-synaptic depolarization and, thereby, reduces Fos expression (grey nulei). When rimonabant (SR141716) is administered after habituation has been established (c), 2-AG can no longer inhibit presynaptic glutamate release, and Fos expression is reinstated to pre-habituation levels.