Published in final edited form as:

Neuroscience. 2013 December 3; 253: . doi:10.1016/j.neuroscience.2013.08.066.

Adolescent chronic mild stress alters hippocampal CB1 receptor-mediated excitatory neurotransmission and plasticity

Christian G. Reich, Gregory R. Mihalik, Anthony N. Iskander, Joshua C. Seckler, and Michael S. Weiss

Program in Psychology, Ramapo College of New Jersey, Mahwah, NJ, 07430

Abstract

Endocannabinoids (eCBs) are involved in the stress response and alterations in eCB signaling may contribute to the etiology of mood disorders. Exposure to chronic mild stress (CMS), a model of depression, produces downregulation of the CB1 receptor (CB1) in the hippocampus of male rats. However, it is unknown how this stress-induced change in CB1 levels affects eCB-mediated neurotransmission. In vitro, field potential recordings from CMS-exposed (21-days) rats were performed to assess the effects of stress on eCB-regulated glutamatergic neurotransmission in/on hippocampal area CA1. We observed that application of the CB1 agonist, WIN 55,212-5 (1 μ M), in stress animals resulted in a ~135% increase in excitatory neurotransmission, whereas CB1 activation in non-stress animals leads to a ~30% decrease. However, during blockade of GABA(A) neurotransmission with picrotoxin, CB1 activation yielded a ~35% decrease in stress animals. These findings indicate that CMS does not directly affect glutamatergic neurotransmission. Rather, CMS sensitizes CB1 function on GABAergic terminals, leading to less inhibition and an increase in excitatory neurotransmission. This finding is reinforced in that induction of weak LTP is enhanced in CMS-exposed animals compared to controls and this enhancement is CB1-dependent. Lastly, we observed that the LTP-blocking property of WIN 55,212-5 shifts from being glutamate-dependent in non-stress animals to being GABA-dependent in stress animals. These results effectively demonstrate that CMS significantly alters hippocampal eCB-mediated neurotransmission and synaptic plasticity.

Keywords

CB1 receptor; LTP; depression; hippocampus

1. Introduction

Mood disorders such as major depressive disorder are serious mental illnesses that affect approximately 20% of Americans (Kessler et al., 2010). The cannabinoid receptor, CB1 and its ligands, the endocannabinoids (eCBs), are intricately involved in the stress response (Gorzalka and Hill, 2011; Hill et al., 2010) and are therefore putative contributors to the etiology of depressive disorders. Martin et al., (2002) first demonstrated that mutant mice deficient in CB1 receptors show an enhanced vulnerability to the depressive effects of a

© 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

Corresponding Author: Christian G. Reich, Ph.D., Program in Psychology, Ramapo College of New Jersey, Mahwah, NJ 07430, U.S.A., Tel: 201-684-7742, creich@ramapo.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.

chronic mild stress protocol (CMS), a valid preclinical model of depression (Willner, 2005). Butressing this finding, three-week exposure to a similar chronic mild stress protocol produced a ~50% reduction in CB1 levels in several limbic structures including the hippocampus (Hill et al., 2005, 2008b; Reich et al., 2009). Exposure to both chronic mild stress and chronic restraint stress consistently reduces the endocannabinoid/endovanilloid, Anandamide (AEA), in the hippocampus, amygdala, striatum, medial prefrontal cortex and hypothalamus (Gorzalka and Hill, 2011). Conversely, the other major endocannabinoid (eCB), 2-arachidonoyl-glycerol (2-AG), is transiently enhanced by chronic or acute restraint stress but is either reduced or not affected by chronic mild stress (Gorzalka and Hill, 2011; Wang et al., 2012). This differential modulation of eCBs is intriguing considering that 2-AG mediates depolarization-induced-suppression-of-inhibition (DSI), a short-term suppression of GABA release caused by eCB signaling (Kano et al., 2009), whereas AEA is involved in the tonic activation of CB1 (Kim and Alger, 2010). Indeed, chronic stress is capable of impairing both short and long-term eCB synaptic plasticities in the striatum, hypothalamus, nucleus accumbens and hippocampus (Rossi et al., 2008; Wamsteeker et al., 2010; Wang et al., 2010; Hu et al., 2011), although 10 days of chronic restraint stress enhances eCB signaling in the amygdala (Patel et al., 2009; Sumislawski et al., 2011). Notably, these stress effects on eCB function are mimicked by administration of chronic corticosterone to animals (Hill et al., 2008a; Bowles et al., 2011) and blocked by antagonizing glucocorticoid receptors (Gorzalka and Hill, 2011). Thus, stress appears to modulate eCB signaling via the Hypothalamic-Pituitary-Adrenal-Axis (HPA), which is responsible for regulating glucocorticoid levels (stress hormones) in response to environmental stressors.

Twenty-one days of chronic restraint stress (CRS) impairs DSI in the hippocampus of adult male animals (Hu et al., 2011); providing evidence that chronic stress is capable of affecting GABAergic CB1 function. CRS is a validated method to increase HPA activity to achieve hypersecretion of glucocorticoids; however this homotypic stress usually produces habituation of the HPA response (Vyas et al., 2002; Hill et al., 2010). In contrast, CMS paradigms that use heterotypic stressors tend to produce non-habituating HPA responses. CMS is considered a valid animal model of depression partly due to the fact that incorporating multiple stressors minimizes HPA habituation (Willner, 2005). Furthermore, Vyas et al. (2002) observed that chronic restraint stress produces more severe dendritic atrophy than CMS in the hippocampus. They also reported that CRS, but not CMS, causes dendritic hypertrophy in the amygdala. These differences in the two most common chronic stress protocols suggest that CMS may result in different effects on the endocannabinoid system. This highlights the necessity to investigate stress-modulation of eCBs with both methods.

Given that the aforementioned CMS-induced regulation of CB1 was studied using either western immunoblotting (Hill et al., 2005; Reich et al., 2009) or competitive binding assays (Hill et al., 2005; Hill et al., 2008a), it is unknown whether these differences are represented in the general CB1 population or in a particular sub-population. Moreover, it remains unclear how these stress-induced changes affect synaptic physiology in the hippocampus. For example, in the CA1 region of the rodent hippocampus, CB1 resides primarily on the nerve terminals of cholecystokinin (CCK)-containing interneurons (Freund, 2003) and at the glutamatergic synapse between the CA3 Schaffer collateral/commissural terminals and CA1 pyramidal cells (Domenici et al., 2006; Kawamura et al., 2006). Although, compared to the CCK-GABA cells, the glutamatergic-CA3 cells contain a much lower density of CB1 (Domencini et al., 2006; Kawamura et al., 2006). Thus, the primary purpose of this study is to assess if CMS differentially affects CB1-mediated glutamatergic and GABAergic neurotransmission.

In a recent study from our lab, CMS exposure during adolescence selectively enhanced hippocampal-dependent fear conditioning in male rats. This enhancement in aversive learning was prevented by exogenous CB1 activation (Reich et al., 2013). These findings are consistent with previous observations that peri-adolescent/adolescent exposure to chronic stress alters CB1 signalling in the hypothalamus, amygdala and prefrontal cortex (Wamsteeker et al., 2010; Lee and Hill 2012). To date, these are the only three studies that directly address the effects of adolescent stress on the eCB system. This is surprising, given that adolescence is a period of neuronal maturation that is highly vulnerable to stress (Spear, 2000; Lee and Gorzalka, 2012). Exploring the relationship between stress and eCB signalling during this developmental period may assist in elucidating the etiology of stress-related pathologies such as Major Depressive Disorder and PTSD. Using hippocampal fEPSP recordings from adolescent male animals exposed to CMS, we hypothesized that CMS-downregulation of CB1 would impair function on both glutamatergic and GABAergic synapses. However, we observed that CMS does not affect glutamate-CB1 function directly but does increase glutamatergic neurotransmission through a sensitized GABA-CB1 function. This enhanced CB1 function on GABAergic neurotransmission also facilitates LTP induction to weak theta-burst stimulation and shifts the dependency of LTP inhibition by CB1 agonists from glutamatergic to GABAergic neurotransmission.

2. Materials and Methods

2.1 Subjects

Male Sprague-Dawley rats (Charles River, Boston, MA) were group-caged (3 per cage) and allowed to acclimate for 5–7 days prior to experimental testing. All animals were 40–45 days old at the beginning of experimental procedures and maintained on a 12-h/12-h light–dark cycle with lights on at 8:00 a.m. Food and water were available *ad libitum* in the home cages, unless otherwise noted. All experimental procedures were carried out in accordance with protocols established by the Institutional Animal Care and Use Committee of the Ramapo College of New Jersey.

2.2 Chronic Mild Stress Protocol (CMS)

Animals were subjected to either the CMS protocol or the non-stress protocol (handled daily). Each day, 1–3 stressors were administered according to a set schedule. The complete regimen lasted 7 days/wk for 3 wks. This protocol is modeled after Willner (2005) in that no individual stressor is considered severe but that the unpredictability of the protocol constitutes much of the stress. The stressors were: 1) 5 min swim in 20° C water, 2) cage rotation (social stress), 3) 18-hr social isolation with damp bedding, 4) 14-hr food deprivation, 14-hr water deprivation or 14-hr food and water deprivation, 5) 30 min physical restraint, 6) 30 min strobe light exposure and 7) 3-hr cage tilt. In previous studies, we observed that this particular CMS protocol resulted in decreased body weight gain in both male and female animals and reduced sucrose preference in male animals (Reich et al., 2009). These effects are in accordance with the published behavioral effects of CMS protocols (Hill et al, 2005; Willner, 2005).

2.3 Electrophysiology

Animals were deeply anesthetized with halothane and decapitated from approximately 10:00 am–11:00 am. Stress animals were sacrificed 24–72 hours following the last stressor. The brain was rapidly removed and hippocampi dissected. Transverse hippocampal slices, 400 μ m thick, were cut on a Vibrotome (Leica-Microsystems). Slices were kept in a holding chamber at room temperature at the interface of artificial cerebrospinal fluid (ACSF) and a humidified 95/5% O₂/CO₂ atmosphere for >1 hr. The slices were then transferred to a submerged recording chamber and perfused with warm (30° C) ACSF: (mM), NaCl, 120;

KCl, 3; MgSO₄, 2; NaH₂PO₄, 1; NaHCO₃, 25; CaCl₂, 2.5; and glucose, 10 and saturated with 95% O₂-5% CO₂ (pH 7.4). Field potentials (fEPSPs) were recorded from stratum radiatum of CA1 with glass microelectrodes (tip diameter ~4–8 μ m) filled with extracellular saline. Stimuli were delivered via a bipolar stimulating electrode located in stratum radiatum, between CA3 and CA1. Signals were recorded with an amplifier (Model 773, WPI Inc. or Model IE-251A, Warner Instruments Inc.), digitized at 10 kHz with an AD interface (Digidata 1440A, Axon Instruments or PCI-6259, National Instruments) and analyzed with either pClamp 10.0 (Axon Instruments) or WinLTP software. Only fEPSPs that could achieve or exceed an initial amplitude of 1.0 mV were recorded. Stable baseline responses (10–15 min) were recorded prior to experimental manipulation. During pharmacological experiments, fEPSPs were adjusted to ~0.8 mV prior to beginning a baseline. Following the baseline period, drugs were bath-applied for 30–45 min or delivered continuously throughout the entire experiment. Synaptic plasticity experiments began with fEPSPs adjusted to ~0.5 mV and involved delivering a weak theta-burst stimulation (TBS; 10 bursts of 5 stimuli, 100 Hz within burst, 200 ms interburst interval) following the baseline period.

2.4 Data Analysis

The initial slope of the fEPSP (mV/ms) was measured within the first two milliseconds of the response immediately after the negative peak of the fiber volley. To standardize responses across experimental conditions, all slope measurements within a given experiment were divided by the average baseline response (10 or 15 min). Then, individual experiments within each condition were averaged across each time point. For statistical analyses, the mean baseline value (last 5 min; fifteen consecutive responses) and the mean experimental value (last 5 min; fifteen consecutive responses) were computed. Statistical comparisons were performed using one-way ANOVAs, repeated measures MANOVAs and student *t*-tests, *p* = 0.05 (SPSS).

2.5 Pharmacological Treatment

WIN 55,212-5 (1 μ M), AM251 (3 μ M) and Picrotoxin (50 or 100 μ M) were dissolved in dimethyl sulfoxide (DMSO) and prepared from stock solutions. All drugs were diluted in ACSF (1:1000) to achieve final concentrations. Final DMSO concentrations in extracellular saline did not exceed 0.1%. All drugs were purchased from Tocris Cookson Inc. (St. Louis, Mo.)

3. Results

3.1 CB1 receptor activation increases excitatory neurotransmission in CMS animals

Consistent with previous studies (Hoffman et al., 2006; Takahashi and Castillo, 2006) thirty minute application of WIN 55,212-5 (1 μ M; WIN) produced a ~33% decrease in CA1 fEPSPs in slices from non-stress animals. Surprisingly, WIN application to slices from CMS-exposed animals resulted in a ~135% increase in fEPSPs (Fig 1a). A two-way repeated measures ANOVA (Group x Time) confirmed: 1) that following thirty minutes of WIN, the stress ($134.96 \pm 1.50\%$) and non-stress ($67.08 \pm 1.02\%$) groups were significantly different ($F(1,16) = 972.31$, $p = 0.000$) and 2) that WIN significantly altered fEPSP responses in both groups compared to baseline responses ($F(1,16) = 530.60$, $p = 0.000$). To confirm the WIN effects were due to CB1 activation, a subset of slices from both stress and non-stress animals were pre-incubated (>1 hr) and continuously perfused with 3 μ M AM251. During WIN application, AM251 prevented both the WIN-induced increase ($134.96 \pm 1.50\%$ vs. $103.52 \pm 2.20\%$; $t(16) = 18.48$, $p = 0.000$) in stress slices and the WIN-induced decrease in non-stress slices ($67.08 \pm 1.02\%$ vs. $100.64 \pm 1.37\%$; $t(16) = 15.06$, $p = 0.000$) (Fig 1b).

In stress animals, Glutamatergic-CB1 activation was isolated from GABAergic-CB1 activation by continuously treating slices with the GABA(A) antagonist, picrotoxin (100 μ M; PTX). During these experiments, application of WIN in the presence of PTX yielded a significant decrease in fEPSP slope ($56.87 \pm 1.56\%$, $p = 0.001$, t-test) compared to baseline responses, whereas AM251 prevented this decrease ($105.00 \pm 1.56\%$, $p = 0.08$; Fig 2). These observations suggest that CB1 physiology at CA1 glutamatergic synapses is not affected by CMS exposure. However, the data permit the hypothesis that CMS sensitizes GABAergic CB1 physiology resulting in a WIN-induced decrease in CCK-GABAergic neurotransmission and a net gain in excitatory neurotransmission. A summary of the WIN experiments is provided in Figure 3a. Note that these experiments were performed in slices (~2–3 per animal) prepared from the same cohort of stress ($n=20$) and non-stress ($n=12$) animals.

A caveat to the above hypotheses is that CMS exposure alters basal neurotransmission and this explains the differential effects of WIN in stress and non-stress animals. Therefore, prior to recording a formal baseline, input-output curves were generated on a subset of slices to test this idea. As shown in Fig 3b, no significant differences in fEPSP amplitudes were observed between slices from stress and non-stress animals ($p = 0.25$, one-way ANOVA), indicating that exposure to CMS did not alter the excitability of these responses.

3.2 CMS enhances LTP induction from a weak TBS protocol

Endocannabinoid (eCB) activation of CB1 is capable of facilitating LTP induction during both short-term (Carlson et al., 2002) and long-term (Chevalleyre and Castillo, 2004) eCB-mediated suppression of CCK-GABAergic neurotransmission. Since our earlier findings suggest that CMS enhances CB1-mediated suppression of inhibition by exogenous activation, we hypothesized that CMS would lead to enhanced LTP induction. To test this idea, we used a weak Theta-Burst Stimulation protocol (Chevalleyre and Castillo, 2004) that resulted in non-saturating, low-level LTP (~125% of baseline) in a preliminary study with non-stress control animals. When delivered to a separate cohort of CMS ($n=18$) and non-stress animals ($n=15$), the weak TBS produced significantly greater LTP in stress compared to non-stress animals ($174.47 \pm 1.8\%$ vs. $134.31 \pm 1.28\%$; $F(1, 28) = 245.64$, $p = 0.000$, Fig. 4a). In the presence of AM251 (3 μ M), TBS still produced significant LTP compared to baseline ($125.21 \pm 1.8\%$, $p = 0.001$, paired t-test) in slices from stress animals; however, as illustrated in Fig 4b, this LTP was significantly lower than in non-AM251 treated slices ($p = 0.000$, student's t-test). These observations indicate that CMS enhances LTP induction (via a weak TBS protocol) in CA1 and that this enhancement is dependent on CB1 activation.

Despite functioning as a LTP gating mechanism, CB1 via exogenous activation also inhibits LTP induction (Nowicky et al., 1987; Collins et al., 1994, 1995; Terranova et al., 1995; Misner and Sullivan, 1999). This inhibition is thought to underlie learning and memory impairments caused by marijuana use (Nowicky et al., 1987; Terranova et al., 1995). Thus, we pre-incubated slices in WIN (1 μ M) to test if exogenous CB1 activation prior to our weak TBS would also impair LTP in both stress and non-stress animals. In the continuous presence of WIN, LTP was blocked in both stress and non-stress slices ($103.20 \pm 1.8\%$ and $96.73 \pm 0.68\%$, respectively, Fig. 5a and 5b). Next, these experiments were repeated in the presence of picrotoxin to determine the relative contribution of glutamate-CB1 and GABA-CB1 to the WIN-LTP inhibition. Since blocking GABAergic neurotransmission results in enhanced LTP (Wigström and Gustafsson, 1986), we used a relatively weak concentration of PTX (50 μ M) to partially block GABA(A) receptors and to mimic CB1-mediated suppression of neurotransmission. Application of PTX prevented the WIN block of LTP in stress slices ($159.57 \pm 1.54\%$, Fig. 5a) but was not as effective in non-stress slices ($107.19 \pm 1.69\%$, Fig 5b). For comparison, Figure 6 summarizes all the LTP experiments. A one-way ANOVA comparing LTP levels among all the experimental conditions was significant ($F(5,$

89) = 734.04, $p = 0.000$), allowing for further analysis between groups. Post-hoc tests revealed that in stress (S) animals, WIN application significantly impaired LTP compared to control LTP (S; $p = 0.000$) and compared to S_WIN_PTX LTP levels ($p = 0.000$). However, S_WIN_PTX was also significantly lower than control LTP ($p = 0.000$). In non-stress (NS) animals, WIN also inhibited LTP compared to control levels ($p = 0.000$). LTP in the NS_WIN_PTX also was significantly different from control LTP ($p = 0.000$), indicating that PTX did not effectively prevent the WIN impairment. Although, the NS_WIN_PTX group was also significantly different from the NS_WIN ($p = 0.000$), showing that a weak level of plasticity is still possible in these conditions. Importantly, LTP in S_WIN and NS_WIN groups were not significantly different ($p = 0.10$), demonstrating that WIN effectively inhibited LTP in both stress and non-stress animals. The observation that PTX only fully prevented the WIN-LTP block in stress animals suggests that the WIN effect shifts from being glutamate CB1-dependent in non-stress animals to GABA-CB1 dependent in CMS animals.

Previous studies observed that CMS protocols impaired (Alvarez et al., 2003) or had no effect on LTP (Holderbach et al., 2007). Differences in LTP induction protocols could account for the discrepancy between those studies and the current one. Although Alvarez et al. used a primed-burst-stimulation protocol and Holderbach et al. used a paired-Theta-Burst protocol, both groups reported >150% LTP in control animals, which is larger than the magnitude of LTP (~135%) observed in the present study. We tested if a stronger TBS protocol (sTBS; 10 bursts of 5 stimuli, 100 Hz within burst, 200 ms interburst interval, *repeated 4 times*) would also result in enhanced LTP in CMS-exposed animals. As seen in Figure 7, sTBS produced robust LTP in non-stress animals that was significantly greater than LTP produced in stress animals. This finding is consistent with Alvarez et al. and several other studies in the literature that show stress (acute or chronic) is capable of impairing LTP. In our hands, this impairment is only observed during intense LTP-inducing stimulation.

4. Discussion

Exposure to various forms of chronic stress alters CB1 receptor expression and signalling in several brain regions including the hippocampus, hypothalamus, prefrontal cortex, ventral striatum, nucleus accumbens and amygdala (Hill et al., 2008b; Reich et al., 2009; Hill et al., 2005; Wamsteeker et al., 2010; Rossi et al., 2008; Wang et al., 2010; Patel et al., 2009; Sumislowski et al., 2011). The present study investigated the effects of CMS, a model of depression, on hippocampal CB1 physiology. We observed several novel findings: 1) exogenous activation of CB1 by WIN (1 μ M) in stress animals resulted in a ~135% increase in excitatory neurotransmission, whereas CB1 activation in non-stress animals leads to a ~30% decrease in glutamatergic neurotransmission, 2) during blockade of GABA(A) neurotransmission, CB1 activation yielded a ~35% decrease in stress animals, 3) induction of weak LTP is enhanced in CMS-exposed animals compared to controls and this enhancement is CB1 dependent and 4) the LTP-blocking property of WIN shifts from being glutamate-dependent in non-stress animals to being GABA-dependent in stress animals.

Consistent with previous findings (Takahashi and Castillo, 2006; Hoffman et al., 2006), in non-stress animals, WIN application reliably decreased excitatory neurotransmission in a CB1-dependent manner. The observed WIN-induced increase in fEPSPs in CMS animals is surprising given that CMS reliably downregulates hippocampal CB1 in adult animals (Hill et al., 2005; Hill et al., 2008b; Reich et al., 2009). We originally hypothesized that WIN should produce less inhibition on glutamate neurotransmission in stress animals because of a decrease in CB1 levels. However, this hypothesis assumes that CMS equally affects all CB1, regardless of residence. Being that CB1 density is ten-fold higher at CCK-GABAergic

terminals than at glutamatergic terminals, this scenario also proposes that CB1 modulation of both terminal types is scaled as an attempt to maintain a homeostatic balance. Thus, an average CMS-induced reduction in the general hippocampal CB1 population, as demonstrated through either western blot or binding analysis (Hill et al., 2005; Hill et al., 2008b; Reich et al., 2009), may not be representative of individual populations (i.e. GABAergic or glutamatergic). Indeed, pharmacological isolation of CB1 function on glutamatergic terminals, via inhibiting GABAergic neurotransmission, results in a similar WIN-induced fEPSP reduction observed in non-stress animals. This suggests that glutamatergic-CB1 function is spared by CMS and is consistent with other reports demonstrating that different stressors modulate CB1 physiology on GABAergic terminals but not glutamatergic in the amygdala and striatum (Rossi et al., 2008; Patel et al., 2009; Sumislawski et al., 2011). However, chronic stress did impair eCB-mediated glutamatergic neurotransmission in both the hypothalamus (Wamsteeker et al., 2010) and in the nucleus accumbens (Wang et al., 2010). Blocking CB1 with AM251 prevents the WIN effects on fEPSP magnitude in all conditions; demonstrating that these effects are CB1 dependent. This argues against the possibility that WIN is acting either on CB2 receptors or other non-CB1 sites in stress animals.

In the current study, the most parsimonious explanation for the WIN-induced increase in fEPSP magnitude in CMS animals is enhanced CB1 function at GABAergic terminals. We posit that this sensitization of CB1 function is compensating for the loss of receptors occurring during CMS exposure (Hill et al., 2005; Hill et al., 2008b; Reich et al., 2009). One possible mechanism for increased CB1 function within a decreased population is an increase in receptor binding affinity (K_d); although changes in hippocampal CB1 K_d were not observed following CMS exposure (Hill et al., 2008b; Hill et al., 2005). K_d properties of a receptor, however, may be independent of ligand-receptor efficacy (Feldman et al., 1997). That is, CMS-induced reductions in CB1 may increase efficacy, resulting in a compensatory gain in function. Lending evidence to this hypothesis, Wang et al., (2012) demonstrated that a single acute stressor (restraint) augmented depolarization-induced-suppression of inhibition (DSI), a short-term endocannabinoid-mediated plasticity of GABAergic neurotransmission in the rat hippocampus. This enhancement in endocannabinoid (eCB) signaling was due to a glucocorticoid (GC)-induced increase in 2-AG levels. Similar increases in eCB signaling and eCB-mediated plasticity are found in the mouse amygdala following 10 days of chronic restraint stress (Patel et al., 2009; Sumislawski et al., 2011). Despite these reports on stress-induced facilitation of eCB signaling, the predominant observation is that stress attenuates eCB signaling and eCB-mediated synaptic plasticity in the striatum, hypothalamus, nucleus accumbens and hippocampus (Rossi et al., 2008; Wamsteeker et al., 2010; Wang et al., 2010; Hu et al., 2011). For example, 21 days of chronic restraint stress impairs DSI in the rodent hippocampus (Hu et al., 2011). Thus, it appears that while stress generally affects the eCB system, the type and duration of stressor may determine the direction of the stress effect on a given brain structure; an important consideration when comparing across studies.

An important caveat in interpreting the current findings is the age of the animals. Rat adolescence, liberally defined as ~PND 28 to ~ PND 55 (Spear, 2000), is marked by a maturational process of neuronal reorganization and refinement. The endocannabinoid system also undergoes significant maturation during this period. Despite reported variations, brain CB1 and endocannabinoid function generally increase during early development, peaking with the onset of adolescence, then decreasing through adolescence and finally stabilizing in early adulthood- PND 70 (Lee and Gorzalka, 2012). In the present study, it is possible that our observations occurred during this late adolescent decline in CB1 levels. In this scenario, an increase in CB1 density, binding affinity or efficacy in response to CMS exposure may explain the proposed gain in GABAergic CB1 function. However, at the time

of electrophysiological experiments, our animals are between PND 62 and PND 70; a period when CB1 levels are stabilizing to adult levels (Lee and Gorzalka, 2012). We chose this age range to approximate mid-adolescence to young adulthood in humans (Spear 2000; Lee and Gorzalka, 2012). Notably, starting with animals ranging from PND 42 to PND 49, Hu et al., (2011) observed a reduction in DSI, following a 21 day CRS protocol. In comparison, changes in hippocampal CB1 were not observed in rats, PND 35–45, subjected to 10 days of CRS; although receptor downregulation was observed in adult animals, PND 75–85 (Lee and Hill, 2012). The latter result is consistent with previous findings with the CMS protocol in adult animals (Hill et al., 2005; Hill et al., 2008b; Reich et al, 2009), suggesting a developmental regulation of stress-endocannabinoid interactions. Interestingly, Lee and Hill (2012) did observe an approximate 30–40% increase in Kd values in both adolescent and adult animals following CRS. Even though they report this change as non-significant, it contrasts with early observations that changes in Kd were not concomitant with decreases in hippocampal CB1 following exposure to CMS (Hill et al., 2008b; Hill et al., 2005). Furthermore, CB1 upregulation was observed following a 40 day recovery period in CRS-exposed animals, despite no apparent change immediately following the stress (Lee and Hill, 2012). The Kd and recovery data suggest that stress-induced plasticity in CB1 function may not be observed as changes in receptor density. Thus, in the present study, enhanced CB1 function (or decreased function – Hu et al., 2011) would not necessarily be inconsistent with a lack of change in overall receptor density (see above discussion on efficacy). However, as previously noted, the type and duration of stress protocols as well as animal age may produce incongruent results and should be considered when cross-interpreting. For example, a 10 day CRS exposure in juvenile rats (PND 21–30), resulted in a desensitization of CB1 on GABAergic terminals in the hypothalamus, but not in the hippocampus (Wamsteeker et al., 2010). These findings agree with Lee and Hill (2012), but are inconsistent with the current study. Wamsteeker et al. used a 5 sec depolarization step (–70 mv to +20 mv) to induce DSI in both the hypothalamus and hippocampus. A reduction in DSI following CRS was observed in the hypothalamus, however, in the hippocampus a 5-sec depolarization step produces maximal DSI with half-maximal DSI occurring between 100 msec and 1.5 sec depending on the type and concentration of exogenous calcium buffers (Lenz and Alger, 1999). Therefore, if stress altered either Kd or efficacy in hippocampal CB1, then a DSI ceiling effect may have occluded these changes.

The enhanced CB1 function at CCK-GABAergic synapses hypothesis is reinforced by our observation that CMS-exposure facilitates induction of weak LTP. This effect is blocked by AM251 which indicates that not only is the LTP enhancement CB1-dependent but is also dependent on the release of eCBs. In non-stress related studies, hippocampal eCB signaling via CB1 is shown to prime LTP induction during either a DSI window (Carlson et al., 2002) or during long-term eCB-mediated suppression of CCK-GABAergic neurotransmission (Chevalyire and Castillo, 2004). Therefore, it is plausible that CMS-induced sensitization of GABAergic CB1 is a compensatory adaptation of the normal hippocampal eCB-LTP gating mechanism that would help to maintain optimal excitatory CA1 output during periods of compromised function (i.e. chronic stress). This gain in excitatory output may provide additional feedback to the HPA during stress (see discussion below) but would also allow increases in hippocampal-dependent cognitive function. One important caveat is that under a more intense LTP-induction protocol, we observed that LTP in stress slices is inhibited. This result agrees with studies showing that chronic stress in different forms impairs LTP in the hippocampus (Pavlidis et al., 2002; Alvarez et al., 2003). This highlights the notion that varying levels and types of synaptic plasticity may serve different physiologies and behaviors. For example, in a recent study, we observed that this same CMS protocol selectively enhances trace (hippocampal-dependent) fear conditioning, but does not affect either contextual fear conditioning, the prototypical hippocampal-dependent fear task, or non-hippocampal fear conditioning (Reich et al., 2013). However, more evasive 21-day

chronic restraint stress protocols lead to increases in both hippocampal and non-hippocampal dependent forms of fear conditioning (Conrad et al., 1999; Cordero et al., 2003; Pêgo et al., 2008) but also impairs hippocampal LTP induction (Pavlidis et al., 2002).

Again, consistent with several previous studies (Misner and Sullivan, 1999; Nowicky et al., 1987; Collins et al., 1994, 1995; Terranova et al., 1995), pre-treatment with a CB1 agonist prevented LTP induction in non-stress animals. Our data also shows that pre-incubation with WIN inhibits LTP in stress animals. The commonly accepted mechanism for this cannabinoid-LTP block is the suppression of glutamate via CB1 activation and this reduction in glutamate leads to hypoactivation of AMPA and subsequently NMDA receptors (Misner and Sullivan, 1996). However, as we demonstrated, WIN application in CMS animals produces a net increase in excitatory neurotransmission via CB1-induced suppression of GABA release. We hypothesized that pre-incubation with WIN in stress slices would result in a LTP-like increase in excitatory neurotransmission that would occlude subsequent LTP induction. If this is the case, then pharmacologically removing more GABAergic inhibition should allow for additional plasticity. Indeed, blocking GABA(A) receptors with picrotoxin rescued LTP in slices from CMS animals. Interestingly, picrotoxin did not rescue LTP in non-stress slices even though blocking GABA did produce slightly-larger LTP in non-stress slices compared to normal non-stress controls. These results indicate that as previously determined, CB1-mediated block of LTP depends on CB1-activation on glutamatergic terminals in non-stress animals, however in CMS-animals, the LTP inhibition shifts to being dependent on CB1-activation on GABAergic terminals (see Fig 8). This, of course, does not rule out that glutamatergic-CB1 activation does not contribute to blocking LTP in stress slices. Collectively, these findings demonstrate how chronic mild stress re-shapes CB1-mediated synaptic physiology and plasticity.

In adult animals, CRS, enhances eCB signaling in the amygdala which leads to habituation of the stress response by increasing inhibition of the HPA axis (Gorzalka and Hill, 2011). In contrast, CMS protocols lead to non-habituating activation of the HPA (Marin et al., 2007; Grippo and Johnson, 2009). The hippocampus contributes to both fast and delayed feedback inhibition of the HPA through a braking mechanism driven by glucocorticoid (GC) receptors (Jankord and Herman, 2008). Hippocampal excitatory output most likely drives GABAergic neurons in the ventral subiculum and thus provides an inhibitory influence on downstream HPA structures (Herman and Mueller, 2006; Jankord and Herman, 2008). Interestingly, acute stress enhances hippocampal DSI, resulting in a transient increase of excitatory hippocampal output from the CA1/subiculum region (Wang et al., 2011). This suggests that eCBs play an essential role in the hippocampal braking mechanism on the HPA axis. Moreover, this DSI enhancement is due to a GC-induced increase in 2-AG levels with a concomitant decrease in AEA levels (Wang et al., 2012); implicating eCB signalling at CCK-GABAergic terminals in the GC- negative feedback loop on HPA activity. This hypothesized braking mechanism could explain why certain types of repeated stress (i.e. unpredictable, heterotypic stressors) result in non-habituating GC responses. That is, following termination of a stressor, the “brake” would inhibit the GC response to sufficiently prevent habituation. This compensatory mechanism would enable an organism to regulate its responses to stress and respond appropriately to threatening and non-threatening stimuli. Thus, the GC-eCB hippocampal “brake” would be adaptively beneficial when encountering an acute or novel stressor; however, when exposed to unpredictable chronic stress, this mechanism could lead to excessive GC levels. While hippocampal HPA-inhibition appears to be mediated by the ventral hippocampus, basal circadian glucocorticoid secretion is regulated by the dorsal hippocampus (Herman and Mueller, 2006; Jankord and Herman, 2008). We observed that CB1 levels were significantly more downregulated in the dorsal compared to the ventral adult male hippocampus (Reich et al., 2009), indicating that CB1 signalling may regulate the glucocorticoid circadian rhythm. Conversely, the

hippocampus can also enhance HPA activity (Herman and Mueller, 2006; Jankord and Herman, 2008), consequently an increase in hippocampal output, as observed here, could increase HPA activation. Dysfunction in any of these mechanisms may lead to prolonged, excessive or improperly-timed glucocorticoid secretion which, in turn, may damage the hippocampus and other HPA associated structures, resulting in stress-related pathologies such as anxiety, depression and PTSD. Thus, further elucidation of how endocannabinoids modulate the hippocampal-regulation of the HPA axis is necessary for understanding the etiology of stress-related mental disorders.

Acknowledgments

Research supported by NIH R15-MH085280-01 to CGR. We thank Amanda Swanson, Cydney Mitchell, William Hagen, Gabriella Serrano, Wendy Levine, Kevin Manz, Christian Siochi and Timor Petrishin for their excellent technical assistance. In addition, we thank Dr. Carlos LaFourcade for a critical reading of this manuscript and Michele Reich for proofreading and editing.

References

- Alfarez DN, Joëls M, Krugers HJ. Chronic unpredictable stress impairs long-term potentiation in rat hippocampal CA1 area and dentate gyrus in vitro. *Eur J Neurosci.* 2003; 17:1928–1934. [PubMed: 12752792]
- Bowles NP, Hill MN, Bhagat SM, Karatsoreos IN, Hillard CJ, McEwen BS. Chronic, noninvasive glucocorticoid administration suppresses limbic endocannabinoid signaling in mice. *Neuroscience.* 2011; 204:83–89. [PubMed: 21939741]
- Carlson G, Wang Y, Alger BE. Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nature Neurosci.* 2002; 5:723–724. [PubMed: 12080342]
- Chevalere V, Castillo PE. Endocannabinoid-mediated metaplasticity in the hippocampus. *Neuron.* 2004; 43:871–881. [PubMed: 15363397]
- Collins DR, Pertwee RG, Davies SN. The action of synthetic cannabinoids on the induction of long-term potentiation in the rat hippocampal slice. *Eur J Pharmacol.* 1994; 259:R7–8. [PubMed: 7982446]
- Collins DR, Pertwee RG, Davies SN. Prevention by the cannabinoid antagonist, SR141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice. *Brit J Pharmacol.* 1995; 115:869–870. [PubMed: 7582512]
- Conrad CD, LeDoux JE, Magariños AM, McEwen BS. Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. *Behav Neurosci.* 1999; 113:902–913. [PubMed: 10571474]
- Cordero MI, Venero C, Krut ND, Sandi C. Prior exposure to a single stress session facilitates subsequent contextual fear conditioning in rats. Evidence for a role of corticosterone. *Hormones and Behavior.* 2003; 44:338–345. [PubMed: 14613728]
- Domenici MR, Azad SC, Marsicano G, Schierloh A, Wotjak CT, Dodt H-U, Zieglgänsberger 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]
- Feldman, Robert S.; Meyer Jerrold, S. QLF. *Principles of Neuropsychopharmacology.* Sunderland, MA: Sinauer; 1997.
- Freund TF. Interneuron Diversity series: Rhythm and mood in perisomatic inhibition. *Trends in Neurosciences.* 2003; 26:489–495. [PubMed: 12948660]
- Gorzalka BB, Hill MN. Putative role of endocannabinoid signaling in the etiology of depression and actions of antidepressants. *Progress in neuro-psychopharmacology & biological psychiatry.* 2011; 35:1575–1585. [PubMed: 2111017]
- Grippe AJ, Johnson AK. Stress, depression and cardiovascular dysregulation: a review of neurobiological mechanisms and the integration of research from preclinical disease models. *Stress.* 2009; 12:1–21. [PubMed: 19116888]

- Herman JP, Mueller NK. Role of the ventral subiculum in stress integration. *Behav Brain Research*. 2006; 174:215–224.
- Hill MN, Carrier EJ, Ho W-SV, Shi L, Patel S, Gorzalka BB, Hillard CJ. Prolonged glucocorticoid treatment decreases cannabinoid CB1 receptor density in the hippocampus. *Hippocampus*. 2008a; 18:221–226. [PubMed: 18058925]
- Hill MN, Carrier EJ, McLaughlin RJ, Morrish AC, Meier SE, Hillard CJ, Gorzalka BB. Regional alterations in the endocannabinoid system in an animal model of depression: effects of concurrent antidepressant treatment. *Journal of neurochemistry*. 2008b; 106:2322–2336. [PubMed: 18643796]
- Hill MN, McLaughlin RJ, Bingham B, Shrestha L, Lee TTY, Gray JM, Hillard CJ, Gorzalka BB, Viau V. Endogenous cannabinoid signaling is essential for stress adaptation. *PNAS*. 2010; 107:9406–941. [PubMed: 20439721]
- Hill MN, Patel S, Carrier EJ, Rademacher DJ, Ormerod BK, Hillard CJ, Gorzalka BB. Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. *Neuropsychopharmacology*. 2005; 30:508–515. [PubMed: 15525997]
- Hoffman AF, Macgill AM, Smith D, Oz M, Carl R. Species and strain differences in the expression of a novel glutamate modulating cannabinoid receptor in the rodent hippocampus. *Eur J Neurosci*. 2006; 22:2387–2391. [PubMed: 16262678]
- Holderbach R, Clark K, Moreau J-L, Bischofberger J, Normann C. Enhanced long-term synaptic depression in an animal model of depression. *Biological Psychiatry*. 2007; 62:92–100. [PubMed: 17141742]
- Hu W, Zhang M, Czéh B, Zhang W, Flügge G. Chronic restraint stress impairs endocannabinoid mediated suppression of GABAergic signaling in the hippocampus of adult male rats. *Brain Research Bulletin*. 2011; 85:374–379. [PubMed: 21527320]
- Jankord R, Herman JP. Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. *Annals of the New York Academy of Sciences*. 2008; 1148:64–73. [PubMed: 19120092]
- Kano M, Ohno-Shosaku T, Hashimoto Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. *Physiological Reviews*. 2009; 89:309–380. [PubMed: 19126760]
- Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, Watanabe M, Ohno-Shosaku T, Kano M. The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J Neurosci*. 2006; 26:2991–3001. [PubMed: 16540577]
- Kessler RC, et al. Age differences in the prevalence and co-morbidity of DSM-IV major depressive episodes: results from the WHO World Mental Health Survey Initiative. *Depression and anxiety*. 2010; 27:351–364. [PubMed: 20037917]
- Kim J, Alger BE. Reduction in endocannabinoid tone is a homeostatic mechanism for specific inhibitory synapses. *Nature Neurosci*. 2010; 13:592–600. [PubMed: 20348918]
- Lee TT-Y, Gorzalka BB. Timing is everything: evidence for a role of corticolimbic endocannabinoids in modulating hypothalamic-pituitary-adrenal axis activity across developmental periods. *Neuroscience*. 2012; 204:17–30. [PubMed: 22015924]
- Lenz RA, Alger BE. Calcium dependence of depolarization-induced suppression of inhibition in rat hippocampal CA1 pyramidal neurons. *J Phys*. 1999; 521(Pt 1):147–157.
- Marin MT, Cruz FC, Planeta CS. Chronic restraint or variable stresses differently affect the behavior, corticosterone secretion and body weight in rats. *Physiology & Behavior*. 2007; 90:29–35. [PubMed: 17023009]
- Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology*. 2002; 159:379–387. [PubMed: 11823890]
- Misner DL, Sullivan JM. Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. *J Neurosci*. 1999; 19:6795–6805. [PubMed: 10436037]
- Nowicky AV, Teyler TJ, Vardaris RM. The modulation of long-term potentiation by delta-9-tetrahydrocannabinol in the rat hippocampus, in vitro. *Brain Research Bulletin*. 1987; 19:663–672. [PubMed: 2830949]

- Patel S, Kingsley PJ, Mackie K, Marnett LJ, Winder DG. Repeated homotypic stress elevates 2-arachidonoylglycerol levels and enhances short-term endocannabinoid signaling at inhibitory synapses in basolateral amygdala. *Neuropsychopharmacology*. 2009; 34:2699–2709. [PubMed: 19675536]
- Pavlidis C, Nivón LG, McEwen BS. Effects of chronic stress on hippocampal long-term potentiation. *Hippocampus*. 2002; 12:245–257. [PubMed: 12000121]
- Pêgo JM, Morgado P, Pinto LG, Cerqueira JJ, Almeida OFX, Sousa N. Dissociation of the morphological correlates of stress-induced anxiety and fear. *Eur J Neurosci*. 2008; 27:1503–1516. [PubMed: 18336570]
- Reich CG, Taylor ME, McCarthy MM. Differential effects of chronic unpredictable stress on hippocampal CB1 receptors in male and female rats. *Behav Brain Research*. 2009; 203:264–269.
- Reich, Christian G.; Iskande, Anthony N.; Weiss, MS. Cannabinoid modulation of chronic mild stress-induced selective enhancement of trace fear conditioning in adolescent rats. *J Psychopharma*. 2013 In Press.
- Rossi S, De Chiara V, Musella A, Kusayanagi H, Mataluni G, Bernardi G, Usiello A, Centonze D. Chronic psychoemotional stress impairs cannabinoid-receptor-mediated control of GABA transmission in the striatum. *J Neurosci*. 2008; 28:7284–7292. [PubMed: 18632932]
- Spear LP. The adolescent brain and age-related behavioral manifestations. *Neuroscience and biobehavioral reviews*. 2000; 24:417–463. [PubMed: 10817843]
- Sumislowski JJ, Ramikie TS, Patel S. Reversible Gating of Endocannabinoid Plasticity in the Amygdala by Chronic Stress: A Potential Role for Monoacylglycerol Lipase Inhibition in the Prevention of Stress-Induced Behavioral Adaptation. *Neuropsychopharmacology*. 2011; 36:2750–2761. [PubMed: 21849983]
- Takahashi, Ka; Castillo, PE. The CB1 cannabinoid receptor mediates glutamatergic synaptic suppression in the hippocampus. *Neuroscience*. 2006; 139:795–802. [PubMed: 16527424]
- Terranova JP, Michaud JC, Le Fur G, Soubrié P. Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212-2: reversal by SR141716 A, a selective antagonist of CB1 cannabinoid receptors. *Naunyn-Schmiedeberg's archives of pharmacology*. 1995; 352:576–579.
- Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J Neurosci*. 2002; 22:6810–6818. [PubMed: 12151561]
- Wamsteeker JJ, 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 Psychopharm*. 2012; 26:56–70.
- Wang W, Sun D, Pan B, Roberts CJ, Sun X, Hillard CJ, Liu Q. Deficiency in endocannabinoid signaling in the nucleus accumbens induced by chronic unpredictable stress. *Neuropsychopharmacology*. 2010; 35:2249–2261. [PubMed: 20664582]
- Wigström H, Gustafsson B. Postsynaptic control of hippocampal long-term potentiation. *Journal de physiologie*. 1986; 81:228–236. [PubMed: 2883309]
- Willner P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology*. 2005; 52:90–110. [PubMed: 16037678]

Research Highlights

Chronic Mild Stress increases hippocampal CA1 fEPSP magnitude during exogenous CB1 activation

In non-stress controls, exogenous CB1 activation produces a decrease in fEPSP magnitude

Chronic Mild Stress increases CB1-dependent LTP-induction by weak theta-burst stimulation

In Chronic Mild Stress animals LTP inhibition with prior CB1 activation is GABA dependent

In non-stress animals LTP inhibition with prior CB1 activation is glutamate dependent

Figure 1. CB1 activation in chronic stress animals enhances CA1 excitatory neurotransmission
a) Application of the CB1 agonist WIN (1 μ M) decreases fEPSP magnitude in non-stress control animals, but increases magnitude in stress animals. b) The CB1 antagonist prevents the WIN effect in both non-stress and stress animals. In both figures, the top row displays raw traces of example experiments and the bottom shows time course of averaged experiments.

Figure 2. Blocking GABA(A) neurotransmission leads to a decrease in CB1-mediated CA1 excitatory neurotransmission in chronic stress animals

Continuous application of the GABA(A) antagonist picrotoxin (100 μM) reverses the WIN-enhancement of fEPSP magnitude in slices from stress animals. Thus, in picrotoxin, WIN produces a similar decrease in glutamatergic neurotransmission as observed in non-stress slices. Application of AM251 prevents this decrease, confirming that WIN is activating CB1.

Figure 3. WIN Summary

a) Bar graph summarizing the WIN experiments. * = significant difference compared to the non-stress group; # = significant difference compared to the stress group; $p < 0.05$. b) fEPSP amplitude vs. stimulation intensity demonstrates that CMS did not affect basal neurotransmission compared to non-stress controls.

Figure 4. Weak TBS stimulation produces enhanced LTP in CMS animals

a) LTP magnitude is significantly enhanced in stress compared to non-stress animals. b) AM51 prevents LTP enhancement in stress animals. Top: Raw traces of sample experiments; Middle and Bottom Left: time course of average experiments; Middle and Bottom Right: summary graph. * = significant differences compared to baseline; # = significant differences between non-stress and stress, and between stress and stress+AM251; $p < 0.05$.

Figure 5. Pre-treatment with WIN blocks LTP induction in both stress and non-stress animals
a) Pre-incubation with WIN impairs LTP in slices from CMS animals and application of picrotoxin (50 μ M) rescues this deficit. b) Pre-incubation with WIN impairs LTP in slices from non-stress animals but application of picrotoxin does not prevent this deficit. Top: Raw traces of sample experiments; Middle and Bottom Left: time course of average experiments; Middle and Bottom Right: summary graph. * = significant differences compared to baseline; # = significant differences compared to either the stress_WIN group or the non-stress_WIN, respectively; $p < 0.05$.

Figure 6. Summary of WIN-LTP experiments

* = significant difference compared to the non-stress group, ^ = significant difference compared to the stress group, # = significant differences between stress and stress_WIN_PTX; $p < 0.05$.

Figure 7. LTP is impaired via Strong TBS stimulation in CMS slices

Time course of averaged experiments showing that LTP magnitude is significantly lower in slices from stress animals compared to non-stress slices.

Figure 8. CMS alters CB1-mediated LTP induction in CA1

The diagram illustrates how the hypothesized changes in CB1 signalling affect the gating of LTP. *Top, Left:* a simplified model of the hippocampal CA1 stratum radiatum during basal (non-stress) conditions). Note that CB1 on both glutamatergic and GABAergic (CCK) terminals and that there is a higher density of receptors on GABAergic compared to glutamatergic terminals. *Bottom, Left:* Following CMS exposure, there is no effect on glutamatergic-CB1 function; although a gain in GABAergic-CB1 function is suggested. This model assumes that CMS also yields a reduction in GABAergic-CB1 density, hence less but larger receptors are illustrated. *Right:* Summary of findings from both basal and stress conditions. Please see text for a more thorough discussion.