Regional Alterations in the Endocannabinoid System in an Animal Model of Depression: Effects of Concurrent Antidepressant Treatment

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Abstract

It has been suggested that disturbances in endocannabinoid signaling contribute to the development of depressive illness; however, at present there is insufficient evidence to allow for a full understanding of this role. To further this understanding, we performed an analysis of the endocannabinoid system in an animal model of depression. Male rats exposed to chronic, unpredictable stress (CUS) for 21 days exhibited a reduction in sexual motivation, consistent with the hypothesis that CUS in rats induces depression-like symptoms. We determined the effects of CUS, with or without concurrent treatment with the antidepressant imipramine (10 mg/kg), on CP55940 binding to the cannabinoid CB_1 receptor; whole tissue endocannabinoid content; and fatty acid amide hydrolase (FAAH) activity in the prefrontal cortex, hippocampus, hypothalamus, amygdala, midbrain and ventral striatum. Exposure to CUS resulted in a significant increase in CB_1 receptor binding site density in the prefrontal cortex and a decrease in CB_1 receptor binding site density in the hippocampus, hypothalamus and ventral striatum. Except in the hippocampus, these CUS-induced alterations in CB_1 receptor binding site density were attenuated by concurrent antidepressant treatment. CUS alone produced a significant reduction in N-arachidonylethanolamine (anandamide) content in every brain region examined, which was not reversed by antidepressant treatment. These data suggest that the endocannabinoid system in cortical and subcortical structures is differentially altered in an animal model of depression and that the effects of CUS on CB_1 receptor binding site density are attenuated by antidepressant treatment while those on endocannabinoid content are not.

Keywords

2-AG; cannabinoid; anhedonia; depression; HPA axis; glucocorticoid

Introduction

Depressive illness is widespread and exhibits a lifetime prevalence rate of 16% (Kessler et al., 2003). Depressive illnesses are characterized by an array of disturbances in emotional behavior, memory, neurovegetative functions and hedonic processing. The neurobiological mechanisms subserving the development, manifestation and treatment of depression are complex, and there is ample evidence that disturbances in monoaminergic signaling,

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glucocorticoid activity and neurotrophic/neuroplastic processes are involved (Ressler and Nemeroff, 2000; Holsboer, 2000; Duman and Monteggia, 2006; McEwen, 2005).

Our understanding of the biochemical processes in depression has been shaped by preclinical work employing animal models of depression. While an unequivocal animal model of depression is not available, several models have demonstrated a high degree of biological and behavioral concordance with major depression in humans (Nestler et al., 2002; O'Neil and Moore, 2003). In particular, the chronic unpredictable stress model (CUS; also referred to as chronic mild stress [CMS]) has been found to have reasonable face validity as a model of depression, largely because this model incorporates etiological factors that are similar to human depression (protracted exposure to mild, but unpredictable stressors) and elicits both biochemical (eg. alterations in monoaminergic receptors and glucocorticoid signaling) and behavioral (eg. anhedonia) responses that parallel those seen in human major depression (Lopez et al., 1998; Willner, 2005; however see Reid et al., 1997; Forbes et al., 1996 for a critique of the model). Accordingly, the CUS model represents an useful tool to examine potential systems involved in the pathophysiology of depression.

There is an increasing evidence to support a role for the endocannabinoid system (ECS) in the neurobiology of depression (Hill and Gorzalka, 2005a; Witkin et al., 2005; Vinod and Hungund, 2006; Mangieri and Piomelli, 2007). The central ECS is composed of a G-protein coupled receptor (CB$_1$ receptor; Howlett, 2002) and two endogenous ligands, *N*-arachidonylethanolamide (anandamide or AEA; Devane et al., 1992) and 2-arachidonoylglycerol (2-AG; Sugiura et al., 1995). There is predominant expression of the CB$_1$ receptor on axon terminals where it inhibits transmitter release via activation of G$_{i/o}$ proteins and subsequent attenuation of axonal calcium influx (Freund et al., 2003). Signaling at the CB$_1$ receptor is elicited by both endocannabinoids, which are likely released from postsynaptic cells by changes in neuronal activity (Chevaleyre et al., 2006; Marsicano and Lutz, 2006). The functional lifespan of these molecules is regulated by hydrolytic enzymes; AEA is exclusively metabolized in the brain by fatty acid amide hydrolase (FAAH), while 2-AG is metabolized primarily by monoacylglycerol lipase, although it is likely that other hydrolytic enzymes, including FAAH, can also inactivate 2-AG in the brain (Ligresti et al., 2005; Hillard, 2000a).

The ECS is present in moderate to high levels in limbic brain regions, such as the prefrontal cortex, hippocampus and amygdala, where neuronal activity is known to be altered in depression (Herkenham et al., 1991; Egertova et al., 2003; Bisogno et al., 1999). Genetic deletion of the CB$_1$ receptor in mice results in a phenotype that is reminiscent of the symptom profile of melancholic depression (reviewed in Hill and Gorzalka, 2005a), such as increased depressive-like and anxiety-like behaviors (Steiner et al., 2008b; Uriguen et al., 2004); perseverance of emotionally aversive memories (Marsicano et al., 2002); enhanced activation and impaired inhibition of the hypothalamic-pituitary-adrenal (HPA) axis (Cota et al., 2007); diminished feeding behavior (Ravinet Trillou et al., 2004) and impaired responsiveness to rewarding stimuli (Sanchis-Segura et al., 2004). Consistent with this hypothesis, at the clinical level, we have recently reported that serum 2-AG concentrations are reduced in women with major depression compared to a matched control group (Hill et al., 2008b) and post-mortem analysis has revealed that the expression of CB$_1$ receptors on glial cells in the anterior cingulate cortex is significantly reduced in depressed individuals (Koethe et al., 2007).

Pharmacological facilitation of the ECS has been shown to produce an antidepressant response alone and to enhance the effects of conventional antidepressants in several rodent models of antidepressant efficacy (Bortolato et al., 2007; Filip et al., 2006; Gobbi et al.,

2005; Hill and Gorzalka, 2005b; Hill et al., 2007b). Further, several modalities known to produce antidepressant effects in humans have been shown to increase endocannabinoid/ CB_1 receptor signaling in the brain, including chronic treatment with the antidepressant desipramine (Hill et al., 2006), repeated electroconvulsive shock treatment (Hill et al., 2007a) and sleep deprivation (Chen and Bazan, 2005). Collectively, these studies support the hypotheses that hypofunctional endocannabinoid signaling contributes to the etiology or symptom spectrum of depressive illness and that increasing endocannabinoid signaling is associated with anti-depressant efficacy.

On the other hand, there is also evidence that hyperfunctional ECS is associated with depression. There is a reliable association between long-term cannabis use and the development of suicidal ideations and depressive illness (Bovasso, 2001; Degenhardt et al., 2003; Lynskey et al., 2004), suggesting that overactive CB_1 receptor signaling promotes the development of depression. CB_1 receptor protein expression, binding site density and signal transduction are up-regulated in the prefrontal cortex of depressed individuals who have committed suicide (Hungund et al., 2004). Similarly, chronic mild stress exposure to rats (Bortolato et al., 2007) and CUS exposure to mice (Hillard et al., 2006) increases CB_1 receptor mRNA in the prefrontal cortex. Both acute and chronic treatments with a CB_1 receptor antagonist elicit antidepressant responses in the forced swim test and chronic mild stress paradigm (Griebel et al., 2005; Shearman et al., 2005; Steiner et al., 2008a; Tzavara et al., 2003). Finally, some interventions that reduce depressive symptoms also down-regulate the ECS; including electroconvulsive shock (Hill et al., 2007a) and chronic treatment with the antidepressant fluoxetine (Oliva et al., 2005). Taken together, these results suggest reducing ECS signaling can alleviate depressive symptoms.

In an attempt to reconcile these apparently discrepant findings, we have previously suggested that the endocannabinoid system could differentially contribute to the symptomatology of depression in a region-specific manner (Hill et al., 2007a). Thus, the aim of the current study is to carry out a regional analysis of the endocannabinoid system in rats exposed to CUS model and to explore the sensitivity of these changes to the antidepressant, imipramine. Given the equivocal nature of the CUS model for evoking "depression"-like symptoms (refer to Willner, 2005; Reid et al., 1997; Forbes et al., 1996) we have included a study of the effects of CUS on sexual motivation, a parameter which represents a measure of natural reward and motivational drive and is often impaired in depression (Williams and Reynolds, 2006). The current data confirm the hypothesis that endocannabinoid signaling is regionally modulated in an animal model of depression, and that imipramine normalizes several, but not all, of these changes.

Methods

Subjects

Male, Long-Evans rats (300 g; 70 days of age) housed in groups of three in triple mesh wire caging maintained at 21 °C, and on a 12 h light/dark cycle, with lights on at 0900 h. All rats were given ad libitum access to Purina Rat Chow and tap water, except during deprivation periods of the stressing protocol (outlined below). In addition to the experimental animals, female, Long-Evans rats were used as stimuli for sexual behavior testing. All females were bilaterally ovariectomized at 3 months of age using standard surgical procedures while anesthetized with 75 mg/kg ketamine hydrochloride and 7 mg/kg xylazine (intraperitoneal). All animal protocols were approved by the Canadian Council for Animal Care and the standards of the Animal Ethics Committee of the University of British Columbia.

Chronic Unpredictable Stress Protocol

For behavioral testing, one cohort of animals were either subjected to 21 days of CUS or acted as cage controls and were handled weekly. At the conclusion of this period, all animals were tested for copulatory behavior.

For biochemical assays, a different cohort of animals was employed with four treatment conditions generated to incorporate concurrent antidepressant treatment: 1) vehicle / no stress; 2) imipramine / no stress; 3) vehicle / CUS; 4) imipramine / CUS. Imipramine was dissolved in 0.9 % saline and was administered via intraperitoneal injection at a dose of 10 mg/kg, and at a volume of 1 ml/kg. Injections were given daily between 1200h–1400h using 26 gauge 1/2" needles. The CUS paradigm employed has been repeatedly utilized by our laboratory for both behavioral and biochemical analysis (Brotto et al., 2001; Hill et al., 2005; Hill and Gorzalka, 2004), and is adapted from the original CMS paradigm (Willner et al., 1987). Despite the fact that the original CMS paradigm was designed as an 8–10 week regimen, a large body of research has demonstrated that exposure of rodents to 14 to 21 days of CUS is sufficient to induce neurobiological alterations that parallel those documented in individuals suffering from major depression (Iredale et al., 1996; Lopez et al., 1998; Ossowska et al., 2001, 2002; Xu et al., 2007). Additionally, concurrent administration of antidepressant agents throughout the duration of a 2–3 week CUS paradigm has also been demonstrated to reliably reverse neurochemical changes evoked by this regimen (Lopez et al., 1998; Ossowska et al., 2001, 2002). The CUS paradigm consisted of 2–3 stressors a day from the following list: 30 min tube restraint; 30 min exposure to social crowding with white noise/stroboscopic illumination; 5 min forced swim; 18 h food and/or water deprivation; 3 h cage rotation; and 18 h social isolation in damp bedding. All stressors were separated by a period of at least 2 h and were applied daily over the 21 day period. For the biochemical assays, all animals were rapidly decapitated in the morning after the 21st day of stress exposure following 18 h of overnight social isolation. Brains were removed and the prefrontal cortex (consisting of medial prefrontal cortex and anterior cingulate cortex), hippocampus, amygdala (consisting of central, basolateral and medial nuclei), hypothalamus, ventral striatum (consisting of the nucleus accumbens) and midbrain (including the cell bodies of monoaminergic nuclei in the raphe, ventral tegmental area and locus coeruleus) were sectioned out as previously described (Hill et al., 2006). Brains were frozen in liquid nitrogen within 5–7 min of decapitation and stored at −80 °C until analysis. Two independent cohorts of tissue were collected. One cohort of tissue (n = 7–8) was used for lipid extraction to determine endocannabinoid ligand content. The other cohort of tissue (n=4–5) was used as the source of membranes for CB_1 receptor binding and FAAH activity assays. Trunk blood was collected upon decapitation for measurement of serum endocannabinoid content.

Sexual Behavior Testing

Females were induced into estrus using 10 µg estradiol benzoate 48 h prior to testing and 500 µg progesterone 4 h prior to testing. Both hormones were received from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in peanut oil and injected subcutaneously at a volume of 0.1 ml.

Prior to testing, males were exposed to an estrus-induced female on five occasions and then screened for sexual activity in two independent sessions. To achieve the criterion for sexual proficiency, male subjects had to ejaculate at least once during both of two 30 min screening sessions with a receptive female. Males that achieved this criterion were randomly assigned to be exposed to CUS or functioned as cage controls. All behavioral testing occurred during the middle third of the light cycle and was performed by trained observers who were blinded to the treatment groups. Testing occurred in cubical Plexiglas (30×30×30 cm) and

cylindrical glass (30 cm diameter×45 cm height) chambers lined with contact bedding. Males were habituated to the chambers for 5 min prior to the beginning of testing. Test sessions began with the presentation of an estrus-induced female to a male in an individual testing chamber. The sexual behavior parameters scored were: frequency of mounts with pelvic thrusting prior to ejaculation, frequency of penile intromissions prior to ejaculation, frequency of ejaculations, latency to initiate mounting behavior, latency to initiate intromitting behavior, ejaculation latency (i.e., the period between the first intromission and the first ejaculation) and the postejaculatory interval (i.e., the period between ejaculation and the first intromission of the next copulatory bout). Stimulus females were rotated between males every 10 min to maintain sexual interest.

Membrane Preparation

Dissected brain sections were homogenized in 10 volumes of 0.32 M sucrose containing 3 mM HEPES (pH 7.5) and 1 mM EDTA. The homogenates were centrifuged at $18,000 \times g$ for 20 min after which the supernatant was rapidly decanted. The remaining pellet, which is the membrane fraction, was resuspended in 1–2 ml TME buffer (50 mM Tris HCl, pH 7.4; 1 mM EDTA and 3 mM MgCl2) containing 1 mM sodium orthovanadate. Protein concentrations were determined by the Bradford method (Bio-Rad, Hercules, CA, USA).

CB$_1$ Receptor Binding Assay

CB$_1$ receptor binding assays were performed using a Multiscreen Filtration System with Durapore 1.2-µM filters (Millipore, Bedford, MA) as described previously (Hillard et al., 1995a). Incubations (total volume = 0.2 mL) were carried out using TME buffer containing 1 mg/mL bovine serum albumin (TME/BSA). Membranes (10 µg protein per incubate) were added to the wells containing 0.25, 0.5, 1.0, or 2.5 nM [^{3}H]CP 55,940. Ten µM Δ^9-tetrahydrocannabinol was used to determine non-specific binding. K$_D$ and Bmax values were determined by nonlinear curve fitting to the single site binding equation using GraphPad Prism (San Diego, CA, USA).

Fatty Acid Amide Hydrolase Activity Assay

FAAH activity was measured as the conversion of AEA to arachidonic acid and ethanolamine by membrane preparations (Hillard et al., 1995b). AEA labeled with [^{3}H] in the ethanolamine portion of the molecule ([^{3}H]AEA; Omeir et al., 1995) was the radiolabeled substrate. Membranes were incubated in a final volume of 0.5 ml of TME buffer (50 mM Tris-HCl, 3.0 mM MgCl$_2$, and 1.0 mM EDTA, pH 7.4) containing 1.0 mg/ml fatty acid-free bovine serum albumin and 0.2 nM [^{3}H]AEA. Isotherms were constructed using eight concentrations of AEA at concentrations between 10 nM and 10 µM. Incubations were carried out at 37°C and were stopped with the addition of 2 ml of chloroform/methanol (1:2). After standing at ambient temperature for 30 min, 0.67 ml of chloroform and 0.6 ml of water were added. Aqueous and organic phases were separated by centrifugation at 1,000 rpm for 10 min. The amount of [^{3}H] in 1 ml each of the aqueous and organic phases was determined by liquid scintillation counting and the conversion of [^{3}H]AEA to [^{3}H]ethanolamine was calculated. The K$_I$ and Vmax values for this conversion were determined by fitting the data to a single site competition equation using Prism. The r^2 value for the goodness of fit of the data to the single site, hyperbolic equation was always greater than 0.9 and typically closer to 0.98.

Endocannabinoid Extraction and Analysis

For analysis of endocannabinoid content, brain regions were subjected to a lipid extraction process as described previously (Patel et al., 2003). Briefly, tissue samples were weighed and placed into borosilicate glass culture tubes containing 2 ml of acetonitrile with 84 pmol

of $[^2H_8]$anandamide and 186 pmol of $[^2H_8]$2-AG for extraction. Tissue was homogenized with a glass rod and sonicated for 30 min. Samples were incubated overnight at −10°C to precipitate proteins, and subsequently centrifuged at $1,500 \times g$. The supernatants were removed to a new glass tube and evaporated to dryness under N_2 gas. The samples were resuspended in 300 µl of methanol to recapture any lipids adhering to the glass tube, and dried again under N_2 gas. Finally, lipid extracts were suspended in 20 µl of methanol, and stored at −80°C until analysis.

Trunk blood collected upon decapitation was allowed to settle for 1 h after which it was centrifuged at $1200 \times g$ for 15 min, following which 1 ml of plasma was extracted from each sample and frozen at −80°C until extraction. Plasma extractions were performed similar to that previously described for human serum (Hill et al., 2008b). All plasma extractions were performed using Bond Elut C18 solid-phase extraction columns (1 ml; Varian Inc, Lake Forest, CA). Plasma samples were thawed and made up to 15% ethanol, to which the internal standards $[^2H_8]$-AEA (16.9 pmol) and $[^2H_8]$-2-AG (46.5 pmol) (Cayman Chemicals, Ann Arbor, MI) were added. Samples were then vortexed and centrifuged at $1000 \times g$ for 4 min. The supernatant was loaded on C18 columns, which had been conditioned with 1 ml redistilled ethanol and 3 ml of double distilled water (ddH$_2$O). The remaining pellet was washed with 100 µl of 15% ethanol and centrifuged again for 3 min. The resulting supernatant was also loaded onto the C18 column. Columns were washed with 5 ml ddH$_2$O and eluted with 1 ml of ethyl acetate. The ethyl acetate layer in the resulting elute was removed and dried under N_2. Lipids in the residual ddH$_2$O phase were extracted by mixing with an additional 1 ml of ethyl acetate, which was added to the original ethyl acetate solution. Once dried, samples were resuspended in 20 µl of methanol and stored at −80°C until analysis.

The contents of the two primary endocannabinoids AEA and 2-AG within lipid extracts in methanol from brain tissue and plasma were determined using isotope-dilution liquid chromatography–mass spectrometry as described previously (Patel et al., 2005a).

Statistics

Comparison of the effects of CUS on parameters of sexual behavior was performed using independent t-tests. Comparison of the effects of CUS exposure and/or imipramine administration on parameters of CB$_1$ receptor binding, FAAH activity and endocannabinoid ligand content in different brain regions were analyzed using a univariate analysis of variance (ANOVA), with stress exposure and drug treatment acting as fixed factors. When applicable, post-hoc analysis of each of these variables in each region was performed using a Tukey's test. Significance was established against an alpha level of 0.05.

Results

The Effects of CUS on Sexual Behavior

Exposure of male rats to CUS resulted in a significant increase in the latency of rats to initiate both mounting [t (12) = 3.41, $p < 0.01$; Table 1] and intromitting [t (12) = 3.67, $p < 0.01$; Table 1] behaviors with a receptive female rat. There was a near significant trend in the ability of CUS to increase the latency required for male rats to achieve ejaculation during copulation [t (12) = 1.87, $p = 0.08$; Table 1] as well as to reduce the total number of ejaculations [t (12) = 1.70, $p = 0.1$; Table 1]. There was no effect of CUS exposure on the frequency of mounts [t (12) = 1.21, $p > 0.05$; Table 1]; intromissions [t (12) = 0.34, $p > 0.05$; Table 1];or the latency required to re-initiate sexual activity following the first ejaculation [t (12) = 0.01, $p > 0.05$; Table 1]. Thus, exposure of male rats to CUS resulted in a significant

diminution of motivation for sexual activity, but did not significantly impair sexual performance once it was initiated.

Effects of CUS and/or Imipramine Treatment on CB_1 Receptor Binding Parameters

In the prefrontal cortex (PFC), the agonist binding site density (B_{max}) of the CB_1 receptor was significantly increased in rats exposed to CUS compared to non-stressed controls [$F (1, 16) = 5.09$, $p < 0.04$; Fig. 1a]; there was no effect of imipramine treatment [$F (1, 16) = 2.77$, $p > 0.05$]; and there was no significant interaction between CUS and imipramine treatment [$F (1, 16) = 2.62$, $p > 0.05$]. The equilibrium dissociation constant (K_d) for [^{3}H]CP55940 binding to the CB_1 receptor was not affected by CUS exposure [$F (1, 16) = 2.31$, $p > 0.05$; Table 2] but was significantly reduced by imipramine compared to saline treated rats [$F (1, 16) = 5.09$, $p < 0.04$]. There was no significant interaction between CUS and imipramine treatment [$F (1, 16) = 0.09$, $p > 0.05$]. Therefore, CUS increased CB_1 receptor binding site density in the PFC and imipramine treatment increased agonist affinity for the CB_1 receptor.

In the hippocampus, CB_1 receptor agonist binding site density was significantly decreased by CUS [$F (1, 15) = 11.51$, $p < 0.005$; Fig. 1b] and was unaffected by imipramine [$F (1, 15) = 0.36$, $p > 0.05$]. There was no significant interaction between the effects of CUS and imipramine treatment on the B_{max} of the CB_1 receptor [$F (1, 15) = 0.40$, $p > 0.05$]. There were no significant effects of CUS [$F (1, 15) = 1.14$, $p > 0.05$; Table 2] or imipramine [$F (1, 15) = 0.46$, $p > 0.05$], or a significant interaction between these two factors [$F (1, 15) = 1.00$, $p > 0.05$], on the K_d of [^{3}H]CP55490 for binding to the CB_1 receptor in the hippocampus. Therefore, the only effect of CUS in the hippocampus was a large reduction in CB_1 receptor binding site density; this change was unaffected by imipramine treatment.

In the hypothalamus, analysis of variance revealed that there was a significant interaction between CUS and imipramine treatment on CB_1 receptor binding site density [$F (1, 15) = 10.44$, $p < 0.01$; Fig. 1c]. Post-hoc analysis of these data indicate that the B_{max} for [^{3}H]CP55940 binding to the CB_1 receptor binding site density was decreased both by CUS ($p < 0.01$) and imipramine treatment ($p < 0.05$); however, when CUS and imipramine were administered together, there was no significant difference in this group relative to control animals ($p > 0.05$). A similar pattern was seen in the effects of CUS and imipramine on the K_d of [^{3}H]CP55940 for the CB_1 receptor in that there was a significant interaction between CUS and imipramine treatment [$F (1, 15) = 5.43$, $p < 0.05$; Table 2]. Post hoc analyses reveal that CUS produced a significant decrease in the K_d of [^{3}H]CP55940 the CB_1 receptor ($p < 0.05$) while there was no effect of imipramine treatment alone ($p > 0.05$). When imipramine and CUS were administered concurrently, there was no difference in the K_d of [^{3}H]CP55940 for binding to the CB_1 receptor relative to control animals ($p > 0.05$). Therefore, CUS both reduces CB_1 receptor binding site density and increases agonist affinity in the hypothalamus. Both of these effects of CUS are reversed by co-treatment with imipramine. Imipramine alone did not affect CB_1 receptor binding parameters in the hypothalamus.

In the ventral striatum, there was a significant interaction between CUS and imipramine treatment on the B_{max} of the CB_1 receptor [$F (1, 15) = 11.03$, $p < 0.01$; Fig. 1f]. Post hoc analyses revealed that both CUS ($p < 0.05$) and imipramine treatment ($p < 0.01$) induced significant reductions in B_{max} that were not seen when both treatments were administered concurrently ($p > 0.05$). The K_d for [^{3}H]CP55940 binding to the CB_1 receptor was not affected by CUS [$F (1, 15) = 0.20$, $p > 0.05$; Table 2] but was significantly reduced by imipramine [$F (1, 15) = 12.17$, $p < 0.01$]. There was no interaction between CUS and imipramine treatment [$F (1, 15) = 0.87$, $p > 0.05$]. In sum, the primary effect of CUS on CB_1 agonist binding in ventral striatum is to reduce the Bmax; an effect that is both mimicked and reversed by co-administration of imipramine.

In the amygdala, there was a significant interaction between CUS and imipramine treatments on the B_{max} of the CB_1 receptor [F (1, 14) = 5.10, p < 0.05; Fig. 1d]; however, post hoc analysis demonstrated that this effect was due to an increase in B_{max} following imipramine treatment (p < 0.05) that did not occur in rats exposed to CUS and imipramine treatment combined (p > 0.05). CUS alone did not affect the Bmax for the CB_1 receptor (p > 0.05). The Kd of [^{3}H]CP55940 binding in the amygdala was not affected by CUS [F (1, 14) = 3.76, p > 0.05; Table 2]; imipramine [F (1, 14) = 1.18, p > 0.05]; nor was there an interaction between the treatments [F (1, 14) = 3.76, p > 0.05]. Therefore, there were no effects of CUS on CB_1 agonist binding in the amygdala; however, imipramine alone increased CB_1 receptor binding site density in control but not CUS treated rats.

Within the midbrain, there was no effect of CUS exposure on CB_1 receptor binding site density [F (1, 16) = 2.04, p > 0.05; Fig. 1e], while imipramine produced a significant reduction in B_{max} [F (1, 16) = 4.54, p < 0.05]. There was no interaction between CUS exposure and imipramine treatment on the B_{max} of the CB_1 receptor [F (1, 16) = 1.43, p > 0.05; Fig. 1f]. Neither CUS [F (1, 16) = 1.49, p > 0.05; Table 2] nor imipramine [F (1, 16) = 1.92, p > 0.05], affected the K_d of [^{3}H]CP55940 for the CB_1 receptor in the midbrain, nor was there a significant interaction between these two treatment regimens on this parameter [F (1, 16) = 2.75, p > 0.05]. Therefore, midbrain CB_1 receptor binding parameters are not affected by CUS; however, imipramine alone decreased CB_1 receptor binding site density.

Effects of CUS and/or Imipramine Treatment on Regional, Tissue Endocannabinoid Contents

CUS produced a significant reduction in AEA content in all brain regions examined (Fig. 2). Imipramine treatment alone had no effect on AEA content in any brain region and there were no significant interactions between CUS and imipramine. Therefore, CUS exerts a global and imipramine-insensitive reduction in AEA throughout the limbic system. ANOVA results for the effects of CUS, imipramine treatment, or both on the tissue content of AEA are as follows: Prefrontal cortex [**CUS × IMI**: F (1, 27) = 0.20, p > 0.05; **CUS**: F (1, 27) = 25.14, p < 0.001; **IMI**: F (1, 27) = 0.56, p > 0.05]; hippocampus [**CUS × IMI**: F (1, 26) = 0.08, p > 0.05; **CUS**: F (1, 26) = 20.86, p < 0.001; **IMI**: F (1, 26) = 0.13, p > 0.05]; hypothalamus [**CUS × IMI**: F (1, 25) = 0.89, p > 0.05; **CUS**: F (1, 25) = 8.41, p < 0.01; **IMI**: F (1, 25) = 0.62, p > 0.05]; ventral striatum [**CUS × IMI**: F (1, 27) = 2.06, p > 0.05; **CUS**: F (1, 27) = 25.28, p < 0.001; F (1, 27) = 0.02, p > 0.05]; amygdale [**CUS × IMI**: F (1, 25) = 0.24, p > 0.05; **CUS**: F (1, 25) = 12.14, p < 0.005; **IMI**: F (1, 25) = 0.12, p > 0.05]; and midbrain [**CUS × IMI**: F (1, 27) = 0.24, p > 0.05; **CUS**: F (1, 27) = 16.18, p < 0.001; **IMI**: F (1, 27) = 2.02, p > 0.05].

In contrast, tissue 2-AG contents were increased in rats exposed to CUS only in the hypothalamus and midbrain. These regional increases were unaffected by imipramine treatment (see Table 3 for both data and ANOVA results).

Effects of CUS and/or Imipramine Treatment on Plasma Endocannabinoid Concentrations

CUS produced a significant increase in plasma AEA content [F (1, 25) = 61.24, p < 0.001; Fig. 3]; there was neither an effect of imipramine [F (1, 25) = 0.36, p > 0.05] nor an interaction between CUS and imipramine treatment [F (1, 25) = 0.14, p > 0.05] on plasma AEA. Plasma 2-AG content was unaffected by CUS exposure [F (1, 25) = 1.27, p > 0.05; Fig. 3] and imipramine treatment [F (1, 25) = 0.22, p > 0.05]. There was also no interaction between CUS and imipramine [F (1, 25) = 0.14, p > 0.05] on plasma 2-AG content.

Effects of CUS and/or Imipramine Treatment on FAAH Activity

The Vmax (see Fig. 4) values for the hydrolysis of AEA by membranes were determined in a region-specific manner. There were no effects of either CUS or imipramine alone on the maximal hydrolytic activity of FAAH in any brain region examined; however, within the ventral striatum and midbrain there was a significant interaction between CUS and imipramine treatment such that the combination of these treatments increased the V_{max} of FAAH in these structures. ANOVA results for the effects of CUS, imipramine treatment, or both on the maximal hydrolytic activity (V_{max}) of FAAH are as follows: prefrontal cortex [**CUS × IMI**: $F (1, 15) = 0.48$, $p > 0.05$; **CUS**: $F (1, 15) = 0.66$, $p > 0.05$; **IMI**: $F (1, 15) = 0.00$, $p > 0.05$]; hippocampus [**CUS × IMI**: $F (1, 16) = 0.52$, $p > 0.05$; **CUS**: $F (1, 16) = 0.41$, $p > 0.05$; **IMI**: $F (1, 16) = 0.08$, $p > 0.05$]; hypothalamus [**CUS × IMI**: $F (1, 14) = 2.13$, $p > 0.05$; **CUS**: $F (1, 14) = 0.59$, $p > 0.05$; **IMI**: $F (1, 14) = 0.09$, $p > 0.05$]; amygdala [**CUS × IMI**: $F (1, 12) = 0.22$, $p > 0.05$; **CUS**: $F (1, 12) = 1.00$, $p > 0.05$; **IMI**: $F (1, 12) = 0.06$, $p > 0.05$]; ventral striatum [**CUS × IMI**: $F (1, 14) = 9.57$, $p < 0.01$; post hoc analysis revealed that CUS/IMI is significantly different ($p < 0.05$) relative to no stress/vehicle, CUS/vehicle and no stress/IMI); midbrain [**CUS × IMI**: $F (1, 16) = 4.76$, $p < 0.05$; post hoc analysis revealed that CUS/IMI is significantly different ($p < 0.05$) relative to no stress/vehicle, CUS/vehicle and no stress/IMI).

The binding affinity of AEA for FAAH (K_m) was also determined in a region specific manner. The K_m of FAAH was not effected by CUS alone in any brain region. Similarly, the K_m of FAAH was not effected by imipramine alone, except in the midbrain. The combination of CUS and imipramine increased the K_m of FAAH in the ventral striatum exclusively (data and ANOVA results in Table 4).

Discussion

There has been considerable debate over the validity of the CUS/CMS model to induce behavioral responses akin to the anhedonia seen in human depression (Forbes et al., 1996; Reid et al., 1997). In animal models, anhedonia is typically defined and measured as a deficit in motivational drive for a normally rewarding behavior (Willner et al., 1992). One of the prominent symptoms of depression is a lack of motivation for rewarding activities, which is frequently manifested as a loss of sex drive (Williams and Reynolds, 2006). In the present study, we found that exposure of male rats to three weeks of CUS resulted in a significant impairment in motivation for sexual activity without affecting performance after sexual activity was initiated. These data are consistent with the hypothesis that CUS results in a deficit in motivation for a natural reward and is a useful model for the studies of the neurochemical changes that accompany anhedonia.

In this study, we found that CUS produced changes in both CB_1 receptor agonist binding site density and in the tissue content of the endocannabinoid ligands. Exposure of rats for 21 days to CUS produced a reduction in the tissue content of AEA in all of the brain regions examined (PFC, hippocampus, hypothalamus, ventral striatum, amygdala and midbrain) compared to group housed control rats. This finding stands in contrast to two earlier studies in which CUS did not affect brain AEA contents (Hill et al., 2005; Bortolato et al., 2007). While the reasons for this discrepancy is not clear, it is likely that methodological differences are responsible. For example, in the present study, rats were sacrificed at the end of the dark cycle while both of the other studies collected brain tissue towards the end of light cycle. AEA exhibits a diurnal rhythm in that AEA levels are higher in the dark phase than in the light phase (Valenti et al., 2004). It is possible that a CUS-induced decrease in tissue AEA is only detectable when overall content is high. Regardless of the mechanism, these results demonstrate that tissue eCB changes in response to interventions such as CUS must be interpreted with caution, particularly when only one time point is examined.

In the present study, the CUS-induced reduction in AEA content was not due to accelerated metabolism, as membranes from CUS exposed rats did not exhibit changes in the hydrolysis of AEA. The widespread nature of this reduction is surprising in light of the generally accepted view that endocannabinoid synthesis/release is driven by local changes in neuronal activity. These data support an alternative hypothesis that AEA content in the brain is regulated more broadly and suggest that one of the functions of AEA is to provide a common set point for CB_1 receptor activation throughout the brain. Interestingly, this effect of CUS was unaltered by concurrent treatment with the tricyclic antidepressant, imipramine. These data argue against the hypothesis that the global change in AEA is secondary to the effects of CUS on monoaminergic signaling.

In addition to the effects of CUS on the ECS, it was also found that imipramine treatment alone exerted significant modulation of specific parameters of the ECS. In particular, imipramine treatment was found to increase CB_1 receptor binding in the amygdala, while reducing CB_1 receptor binding in the midbrain; neither of these effects were seen in animals that were concurrently subjected to CUS. The increase in amygdalar CB_1 receptor binding following imipramine treatment is consistent with prior studies from our group which collectively demonstrate that several treatments which are beneficial to depression, such as electroconvulsive shock and tricyclic antidepressant treatment, increase CB_1 receptor activity in subcortical limbic structures, such as the hippocampus, amygdala and hypothalamus (Hill et al., 2006), 2007a. The functional relevance of these changes to the behavioral effects of antidepressant treatment has yet to be elucidated; however, preclinical studies have demonstrated the CB_1 receptor is required for the behavioral effects of noradrenergic based antidepressants but is dispensable for the behavioral effect of serotonergic based antidepressants (Mato et al., 2007; Steiner et al., 2008a).

Rats exposed to CUS exhibited a significant increase in CB_1 receptor binding site density in the PFC, a finding that is consistent with other reports that CUS/CMS results in a significant increase in CB_1 receptor mRNA in the PFC (Bortolato et al., 2007; Hillard et al., 2006). Therefore, it is likely that the increase in PFC CB_1 receptor agonist binding site density reflects an up-regulation of CB_1 receptor expression within specific neuronal populations in the PFC. However, the identity of the cells which exhibit increased CB_1 receptor expression has yet to be determined and will greatly influence the interpretation of the current data. Additionally, the tissue chunk of PFC which was used for membrane preparations in this study (as well as past studies from our group; Hill et al., 2006, 2007a) was composed of both the anterior cingulate cortex, as well as the ventromedial PFC. Neuroimaging studies in human patients suffering from depression have demonstrated that distinct differences exist in the neural alterations seen in the cingulate cortex and more ventral regions of the PFC (Mayberg, 1997; Brody et al., 2001). Thus, future work examining the effects of CUS on CB_1 receptor expression patterns within the anterior cingulate cortex and the ventromedial PFC is required to determine how these changes may relate to alterations in frontocortical neural activity in depressive illness.

Regardless of these caveats, these data are consistent with clinical studies demonstrating that CB_1 receptor protein expression, binding site density and signal transduction are also increased in the PFC of depressed humans who died by suicide (Hungund et al., 2004; Pazos et al., 2006; Vinod et al., 2005). Recent work has demonstrated that increased endocannabinoid/CB_1 receptor signaling in the PFC can increase voluntary alcohol intake (Hansson et al., 2007), suggesting that increased prefrontal cortical CB_1 receptor binding in depression could be related to the high prevalence of alcoholism associated with this disease, as has previously been suggested (Vinod et al., 2005). Moreover, increases in CB_1 receptor signaling within the PFC enhances the association of aversive cues (Laviolette and Grace, 2005), which suggests that increased CB_1 receptor binding in the PFC in depression

could contribute to the enhancement in aversive emotional memory that often characterizes this disease. On the other hand, CB_1 receptors in the ventromedial PFC mediate the antidepressant actions of CB_1 receptor agonists through trans-synaptic activation of dorsal raphe serotonergic neurons (Bambico et al., 2007), suggesting that increased CB_1 receptor density in the PFC could be a compensatory response that dampens the effects of CUS and/ or depression.

In contrast to the PFC, CUS exposure resulted in a significant reduction in CB_1 receptor agonist binding site density in the hippocampus. This finding is in agreement with other reports that CUS reduces hippocampal CB_1 receptor mRNA and protein expression, binding site density and activation of GDP/GTP exchange (Hill et al., 2005; Perez-Rial et al., 2004; Hillard et al., 2006; Reich et al., 2007) but not with a recent report that CB_1 receptor mRNA was not altered in the hippocampus of rats exposed to 10 weeks of CMS (Bortolato et al., 2007). However, in the latter study, the control rats were also exposed to isolation, and food and water deprivation which are stressors themselves, thus could have caused stress-induced changes in CB_1 receptor expression in the controls that would mask changes in the CUS exposed rats.

The effect of CUS to reduce hippocampal CB_1 receptor expression is likely mediated by increased glucocorticoid secretion since CUS elicits glucocorticoid hypersecretion (Herman et al., 1995; Hill and Gorzalka, 2004; Hill et al., 2005) and 21 days of corticosterone administration, in the absence of stress, elicits a comparable reduction in CB_1 receptor binding and protein expression in the hippocampus (Hill et al., 2008a). Imipramine treatment did not affect hippocampal CB_1 receptor density in either control or CUS rats. These findings are in disagreement with earlier findings that a similar tricyclic antidepressant, desipramine, results in an up-regulation of CB_1 receptor binding in the hippocampus of unstressed rats (Hill et al., 2006); however, this discrepancy may be due to the neurochemical targets of the agents of use as desipramine is known to exert a higher preferential affinity for norepinephrine transport over imipramine, which has greater effects on 5-HT reuptake (Langer and Schoemaker, 1988). While this result does not support a role for hippocampal CB_1 receptor density effects in the mechanism of action of imipramine, it does not rule out a role for these changes in the spectrum of CUS-induced behavioral and neurochemical effects.

Similar to what was seen in the hippocampus, CUS reduced the CB_1 receptor agonist binding site density in the hypothalamus. Several studies have established a critical role of the hypothalamic endocannabinoid system in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis; in particular, endocannabinoid/CB_1 receptor signaling inhibits activation of the HPA axis while loss of CB_1 receptors and CB_1 receptor antagonists enhance HPA axis reactivity (Patel et al., 2004, 2005b; Cota et al., 2003, 2007; Steiner et al., 2008a). Disturbances in the HPA axis, such as overactivity and/or resistance to feedback inhibition, are common in melancholic depression (Wong et al., 2000) and are a consequence of CUS (Herman et al., 1995; Hill et al., 2005; Hill and Gorzalka, 2004). Therefore, the reduction in the CB_1 receptor density in the hypothalamus is consistent with the increased activation of the HPA axis seen following CUS. Normalization of HPA axis dysfunction is correlated with remission of depression (Ribeiro et al., 1993; Greden et al., 1983) and antidepressant therapies can diminish HPA axis responsivity in depressed humans and rats exposed to CUS (Duncan et al., 1996; Lopez et al., 1998; Deuschle et al., 2003; Hill et al., 2006). We reported previously that chronic treatment with the antidepressant, desipramine, increases hypothalamic CB_1 receptor agonist binding site density in unstressed rats, which contributes desipramine's ability to dampen HPA axis activation (Hill et al., 2006). Similarly, the present data demonstrate that concurrent imipramine attenuates the effects of CUS to decrease CB_1 receptor binding site density in the hypothalamus. Taken

together, these and earlier findings are consistent with the hypotheses that reduced CB_1 receptor expression contributes to HPA axis hyper-reactivity following CUS and that anti-depressant-induced reversal of this change contributes to the mechanism by which this class of drugs normalizes HPA function in stressed rodents and, possibly, depressed humans.

The tissue content of 2-AG in the hypothalamus was increased following CUS. We have shown previously that acute stress reduces hypothalamic 2-AG content in mice which correlates with increased HPA axis activation (Patel et al., 2004). Furthermore, we found that repeated exposure to a homotypic stressor resulted in both a significant increase in hypothalamic 2-AG and habituation of HPA axis activation response to the stressor. These data support the hypothesis that elevated 2-AG content in the hypothalamus dampens HPA axis activation; if this is correct, then increased hypothalamic 2-AG following CUS would function to oppose HPA hyperactivity. As discussed above, however, CUS also reduced hypothalamic CB_1 receptor density which would produce the opposite effect on HPA activity. It is possible that the change in 2-AG compensates for the loss of CB_1 receptors; or, alternatively, the increase in 2-AG results in CB_1 receptor down-regulation.

The effects of CUS and imipramine on the CB_1 receptor in the ventral striatum were similar to those in the hypothalamus; CB_1 receptor binding densities were reduced by CUS and partially reversed by concurrent imipramine treatment. Activation of CB_1 receptors in the nucleus accumbens within the ventral striatum increases dopamine release and enhances the rewarding properties of both natural and artificial rewards (Caille et al., 2007; Mahler et al., 2007), suggesting, in turn, that decreased CB_1 receptor signaling in this structure could impair hedonic processing. Anhedonia is a core symptom of major depression, and a behavioral phenomenon typically seen in rodents following CUS/CMS (Willner et al., 1992; Willner, 2005). It has recently been shown that the administration of a FAAH inhibitor during CMS (Bortolato et al., 2007) and to mice exposed to repeated restraint (Rademacher and Hillard, 2007) attenuates the development of anhedonia. Furthermore, the induction of anhedonia in response to repeated restraint stress is associated with a reduction in ECS signaling in the ventral striatum (Rademacher and Hillard, 2007). The current data present the first evidence that CUS-induced anhedonia could be due to local impairments in CB_1 receptor activity within the nucleus accumbens, and that the reversal of this effect by antidepressants (or FAAH inhibition) may, in part, be related to normalization of CB_1 receptor signaling in this region; however, this remains to be experimentally demonstrated.

Surprisingly, an interaction between CUS and imipramine occurred in the ventral striatum and midbrain, such that co-treatment of imipramine and CUS resulted in a robust activation of FAAH activity although neither alone had an effect on activity. These data are interesting and suggest that FAAH activity is sensitive to imipramine only in the context of CUS and only in select brain regions; and, alternatively, that FAAH activity is sensitive to CUS only in the context of imipramine treatment. If these data are interpreted in relationship to the model above that a decrease in ventral striatal AEA content is associated with anhedonia, then they suggest that this effect of imipramine to increase FAAH activity in the presence of CUS is an undesirable side effect of the antidepressant that is countering the clinical efficacy of these drugs. In support of this suggestion, preclinical data demonstrate that administration of a FAAH inhibitor can augment the behavioral effects of imipramine treatment (Filip et al., 2006).

We also examined the effects of CUS on circulating endocannabinoid concentrations. Plasma AEA content was significantly elevated following CUS, while plasma 2-AG was not affected. This profile mirrors the plasma endocannabinoid contents in women diagnosed with minor depression (Hill et al., 2008b). While the functional significance of circulating endocannabinoids is not known, peripheral endocannabinoid signaling is known to be

important for immune and cardiovascular parameters (Hillard, 2000b; Kunos et al., 2000; Klein et al., 2003), both of which are often disturbed in depressive illness (Carney et al., 2002; Kim et al., 2007).

Accumulating evidence implicates the endocannabinoid system in the pathophysiology of depression (Gorzalka et al., in press; Hill and Gorzalka, 2005a; Witkin et al., 2005; Vinod and Hungund, 2006; Mangieri and Piomelli, 2007). The results reported herein support that role and demonstrate significant and region-specific changes in the CB_1 receptor and/or endocannabinoids. CUS in rats, which produces some of the important symptoms of human depression (Willner, 2005), significantly reduced CB_1 receptor binding site density in the hippocampus, hypothalamus and ventral striatum and increased density in the PFC. The increase in PFC CB_1 receptor binding site densities following CUS could represent either a compensatory response to maintain serotonergic firing activity (Bambico et al., 2007) or a driving force in the development of depression (Hungund et al., 2004). Alternately, the reduction of CB_1 receptor signaling in subcortical structures could contribute to the changes in HPA axis drive, hedonic valuation and emotional behavior seen in depression. The fact that AEA is so profoundly reduced, that this effect is insensitive to antidepressant treatment and that antidepressants even appear to activate FAAH in the face of concurrent stress in some brain regions, supports the contention that inhibition of FAAH should be investigated as a pharmacological target for the development of novel antidepressants or adjuncts to conventional antidepressants (Gobbi et al., 2005; Bortolato et al., 2007; Hill et al., 2007b).

Abbreviations

CUS	(chronic unpredictable stress)
CMS	(chronic mild stress)
AEA	(*N*-Arachidonylethanolamide)
2-AG	(2-arachidonoylglycerol)
FAAH	(fatty acid amide hydrolase)
PFC	(prefrontal cortex); (hypothalamic-pituitary-adrenal)

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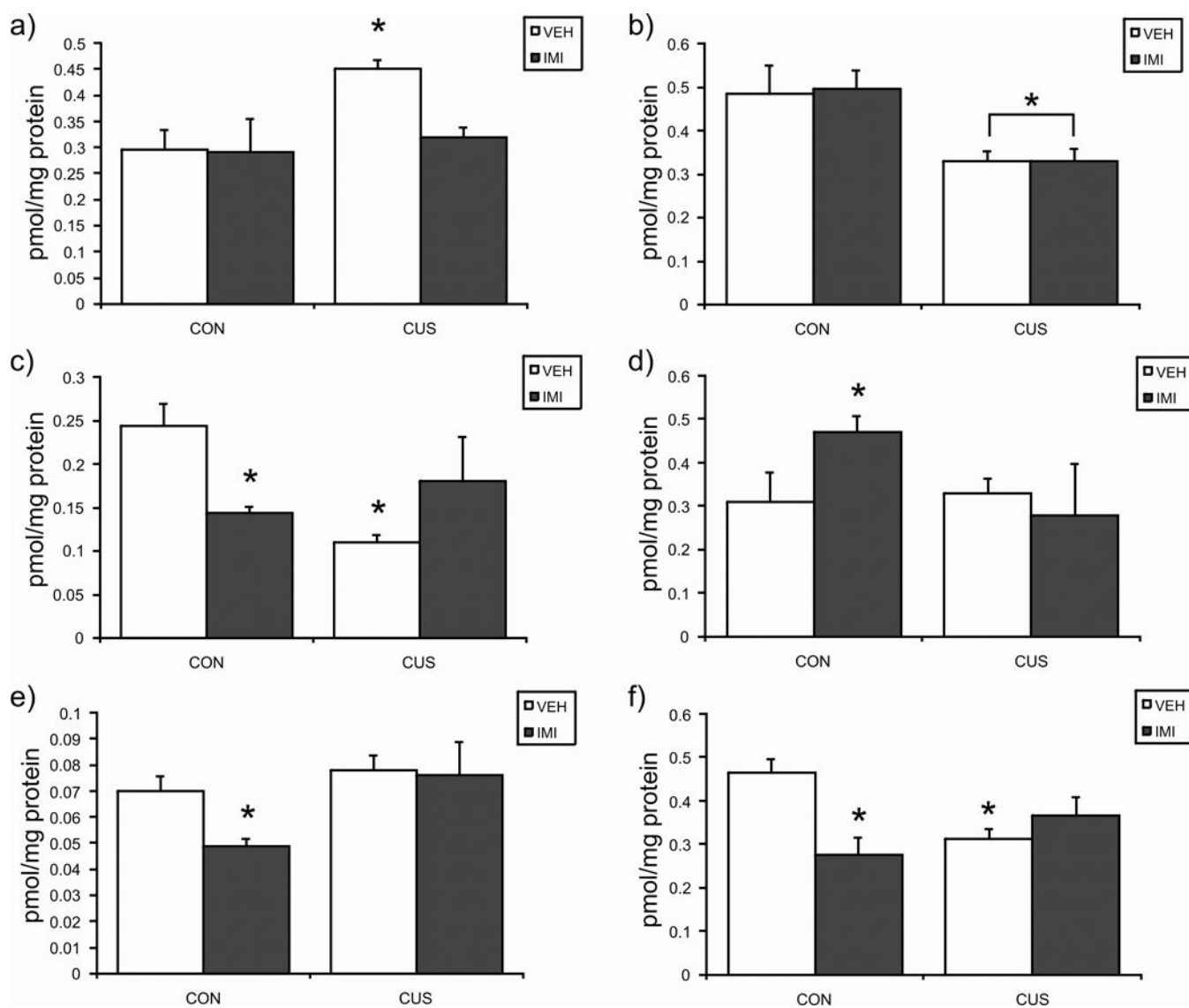
Figure 1.
The effects of exposure to 21 days of chronic unpredictable stress (CUS), imipramine (10 mg/kg; IMI) administration, or both regimens combined, on the maximal binding (B_{max}) of [^{3}H]CP55940 to the cannabinoid CB_1 receptor in the a) prefrontal cortex; b) hippocampus; c) hypothalamus; d) amygdala; e) midbrain; f) ventral striatum. Values are denoted as means ± SEM. Significant differences from non-stressed (CON), vehicle (VEH) treated animals ($p < 0.05$) denoted by *. For all treatment conditions, n = 4–5.

Figure 2.
The effects of exposure to 21 days of chronic unpredictable stress (CUS), imipramine (10 mg/kg; IMI) administration, or both regimens combined, on the tissue content of the endocannabinoid ligand anandamide (AEA) in the a) prefrontal cortex; b) hippocampus; c) hypothalamus; d) amygdala; e) midbrain; f) ventral striatum. Values are denoted as means ± SEM. Significant differences\between groups (p < 0.05) denoted by *. For all treatment conditions are, = 7–8.
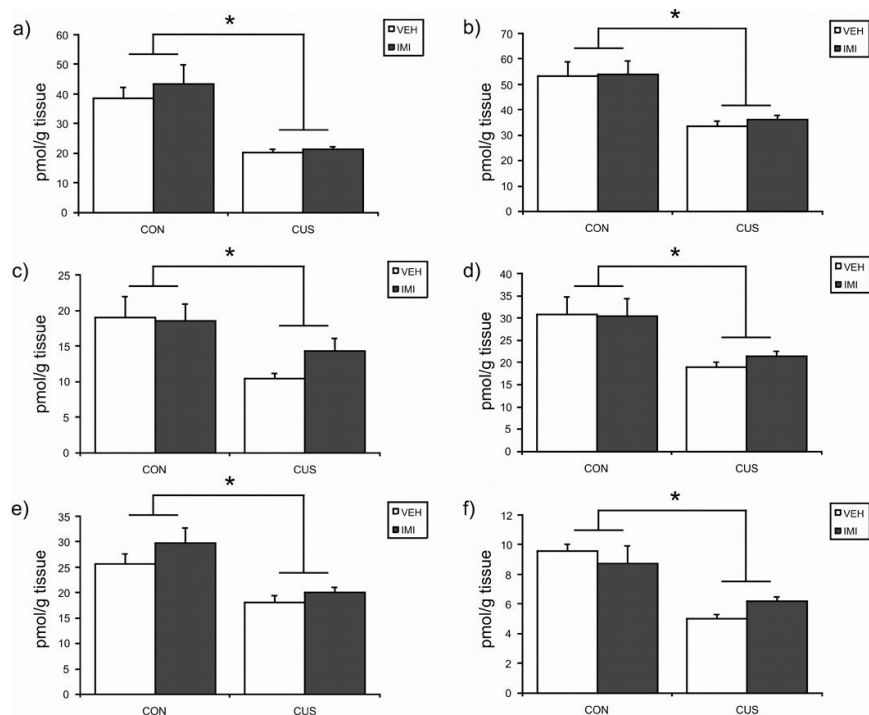
Figure 3.
The effects of exposure to 21 days of chronic unpredictable stress (CUS), imipramine (10 mg/kg; IMI) administration, or both regimens combined, on the plasma content of the endocannabinoids anandamide (AEA; left panel) and 2-arachidonylglycerol (2-AG; right panel). Values are denoted as means ± SEM. Significant differences from non-stressed (CON), vehicle (VEH) treated animals (p < 0.05) denoted by *. For all treatment conditions, n = 6–8.
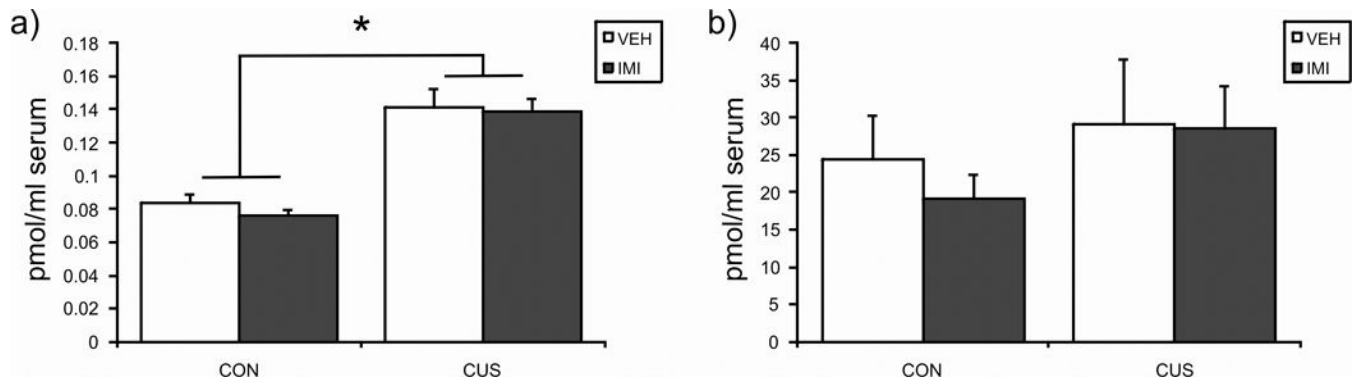
Figure 4.
The effects of exposure to 21 days of chronic unpredictable stress (CUS), imipramine (10 mg/kg; IMI) administration, or both regimens combined, on the the maximal hydrolysis of anandamide by fatty acid amide hydrolase (V_{max}) in the a) prefrontal cortex; b) hippocampus; c) hypothalamus; d) amygdala; e) midbrain; f) ventral striatum. Values are denoted as means ± SEM. Significant differences from non-stressed (CON), vehicle (VEH) treated animals ($p < 0.05$) denoted by *. For all treatment conditions, n = 4–5.
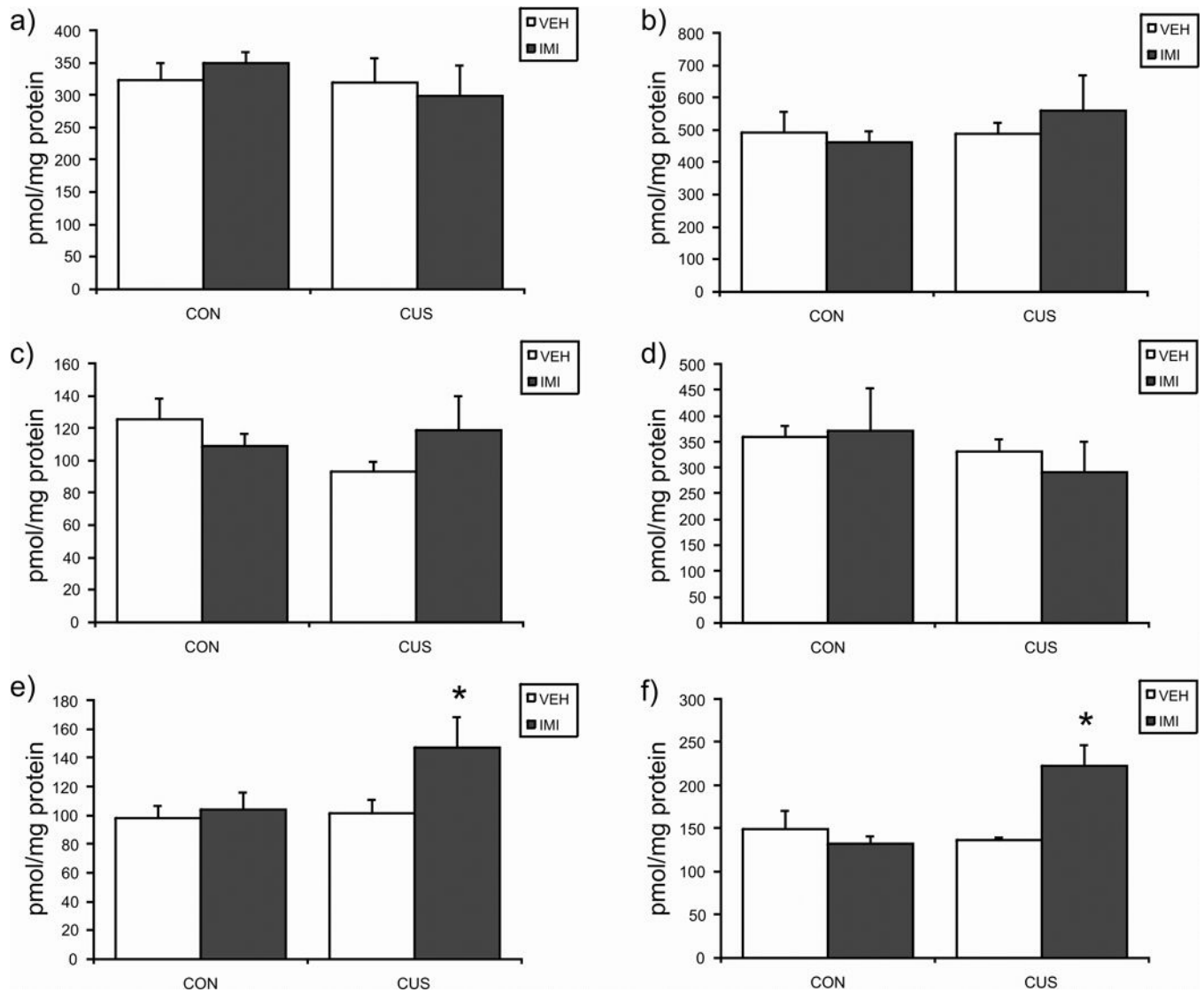
Table 1

The effects of exposure to chronic, unpredictable stress (CUS) on parameters of male sexual behavior

Exposure of male rats to 21 days of CUS resulted in a significant impairment in sexual motivation as demonstrated by the significant increase in the latency to engage in sexual activity with a receptive female rat. The specificity of these effects are demonstrated by the fact that sexual performance itself was not otherwise compromised by CUS exposure. Significant differences from control animals ($p < 0.05$) denoted by *. Both treatment conditions are n = 7. Data are presented as mean values +/− SEM.

	Control	CUS
Mounts:	5.86 +/− 1.18	8.29 +/− 1.63
Intromissions:	11.57 +/− 0.95	12.29 +/− 1.26
Ejaculations:	2.00 +/− 0.31	1.29 +/− 0.29
Mount Latency (s):	19.43 +/− 6.26	68.57 +/− 12.97*
Intromission Latency (s):	26.57 +/− 7.39	80.57 +/− 12.72*
Ejaculation Latency (s):	303.43 +/− 18.52	358.00 +/− 22.51
Post Ejaculatory Interval (s):	311.71 +/− 12.29	311.43 +/− 16.27

Table 2

The effects of exposure to chronic, unpredictable stress (CUS) and/or concurrent treatment with the antidepressant imipramine (IMI; 10 mg/kg) on the dissociation constant (K_d) of ^{3}H-CP55940 from the cannabinoid CB_1 receptor

Exposure of male rats to CUS for 21 days resulted in a significant reduction in the binding affinity (K_d) of the cannabinoid CB_1 receptor in the hypothalamus, and this effect was prevented by concurrent IMI treatment. IMI treatment for 21 days reduced the K_d of the CB_1 receptor in the prefrontal cortex and the ventral striatum. Significant differences from non-stressed (CON), vehicle (VEH) treated animals ($p < 0.05$) denoted by *. For all treatment conditions, n = 4–5. Data are presented as mean nM of ^{3}H-CP55940 +/− SEM.

	CON/VEH	CUS/VEH	CON/IMI	CUS/IMI
Prefrontal Cortex	0.61 +/− 0.14	0.83 +/− 0.10	0.38 +/− 0.15*	0.52 +/− 0.08
Hippocampus	0.60 +/− 0.11	0.59 +/− 0.11	0.79 +/− 0.15	0.55 +/− 0.06
Hypothalamus	1.54 +/− 0.15	0.57 +/− 0.07*	1.02 +/− 0.06	1.22 +/− 0.45
Ventral Striatum	0.65 +/− 0.08	0.55 +/− 0.12	0.34 +/− 0.05*	0.37 +/− 0.02*
Amygdala	0.56 +/− 0.06	0.56 +/− 0.03	0.95 +/− 0.19	0.49 +/− 0.12
Midbrain	0.40 +/− 0.08	0.25 +/− 0.05	0.25 +/− 0.04	0.27 +/− 0.04

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Table 3

The effects of exposure to chronic, unpredictable stress (CUS) and/or concurrent treatment with the antidepressant imipramine (IMI; 10 mg/kg) on the tissue content (nmol/g tissue) of the endocannabinoid 2-arachidonylglycerol (2-AG)

</div>

Exposure of male rats to 21 days of CUS resulted in a significant increase in 2-AG content within the hypothalamus and the midbrain, neither of which was reversed by concurrent IMI treatment. Significant differences from non-stressed (CON), vehicle (VEH) treated animals ($p < 0.05$) denoted by *. For all treatment conditions, $n = 7–8$. Data are presented as mean nmol/g tissue +/− SEM.

	CON/VEH	CUS/IMI	CON/VEH	CUS/IMI
Prefrontal Cortex	4.35 +/− 0.29	5.16 +/− 0.14	5.60 +/− 0.45	5.63 +/− 0.52
CUS × IMI $F (1, 27) = 0.82$, $p > 0.05$; **CUS** $F (1, 27) = 0.93$, $p > 0.05$; **IMI** $F (1, 27) = 3.93$, $p > 0.05$				
Hippocampus	6.37 +/− 0.64	6.11 +/− 0.45	6.82 +/− 0.69	7.04 +/− 0.59
CUS × IMI $F (1, 26) = 0.08$, $p > 0.05$; **CUS** $F (1, 26) = 0.00$, $p > 0.05$; **IMI** $F (1, 26) = 1.28$, $p > 0.05$				
Hypothalamus	8.25 +/− 0.96	12.92 +/− 0.60*	8.38 +/− 0.76	13.56 +/− 0.98*
CUS × IMI $F (1, 25) = 0.10$, $p > 0.05$; **CUS** $F (1, 25) = 35.76$, $p < 0.001$; **IMI** $F (1, 25) = 0.22$, $p > 0.05$				
Ventral Striatum	5.81 +/− 0.77	6.26 +/− 0.45	4.71 +/− 0.27	5.68 +/− 0.26
CUS × IMI $F (1, 27) = 0.32$, $p > 0.05$; **CUS** $F (1, 27) = 2.41$, $p > 0.05$; **IMI** $F (1, 27) = 3.33$, $p > 0.05$				
Amygdala	9.57 +/− 0.68	8.84 +/− 1.04	7.91 +/− 0.94	10.60 +/− 1.08
CUS × IMI $F (1, 25) = 3.00$, $p > 0.05$; **CUS** $F (1, 25) = 0.98$, $p > 0.05$; **IMI** $F (1, 25) = 0.00$, $p > 0.05$				
Midbrain	4.00 +/− 0.31	5.71 +/− 0.44*	5.02 +/− 0.40	5.73 +/− 0.19*
CUS × IMI $F (1, 27) = 2.03$, $p > 0.05$; **CUS** $F (1, 27) = 11.89$, $p < 0.005$; **IMI** $F (1, 27) = 2.20$, $p > 0.05$				

Table 4

The effects of exposure to chronic, unpredictable stress (CUS) and/or concurrent treatment with the antidepressant imipramine (IMI; 10 mg/kg) on the binding affinity (K_m) of fatty acid amide hydrolase for anandamide

Exposure of male rats to 21 days of CUS did not modify the binding affinity of fatty acid amide hydrolase (FAAH) for anandamide. Administration of IMI alone increased the K_m of FAAH, exclusively in the midbrain. However, the joint administration of CUS and IMI resulted in an increase in the Km of FAAH in the ventral striatum and the midbrain. Significant differences from non-stressed (CON), vehicle (VEH) treated animals ($p < 0.05$) denoted by *. For all treatment conditions, n = 4–5. Data are presented as mean nM of ^{3}H-arachidonoylethanolamide +/− SEM.

	CON/VEH	CUS/VEH	CON/IMI	CUS/IMI
Prefrontal Cortex	0.61 +/− 0.08	0.65 +/− 0.09	0.65 +/− 0.10	0.46 +/− 0.05
CUS × IMI: $F_{(1, 15)} = 1.92$, $p > 0.05$; **CUS** $F_{(1, 15)} = 0.68$, $p > 0.05$; **IMI** $F_{(1, 15)} = 0.80$, $p > 0.05$				
Hippocampus	0.64 +/− 0.12	0.69 +/− 0.08	0.80 +/− 0.28	0.71 +/− 0.10
CUS × IMI $F_{(1, 16)} = 0.17$, $p > 0.05$; **CUS** $F_{(1, 16)} = 0.02$, $p > 0.05$; **IMI** $F_{(1, 16)} = 0.28$, $p > 0.05$				
Hypothalamus	0.62 +/− 0.10	0.42 +/− 0.05	0.44 +/− 0.03	0.55 +/− 0.12
CUS × IMI $F_{(1, 14)} = 2.81$, $p > 0.05$; **CUS** $F_{(1, 14)} = 0.20$, $p > 0.05$; **IMI** $F_{(1, 14)} = 0.07$, $p > 0.05$				
Ventral Striatum	0.37 +/− 0.05	0.37 +/− 0.06	0.37 +/− 0.04	0.69 +/− 0.08*
CUS × IMI $F_{(1, 14)} = 7.32$, $p < 0.02$; **CUS** $F_{(1, 14)} = 2.11$, $p > 0.05$; **IMI** $F_{(1, 14)} = 1.90$				
Amygdala	0.81 +/− 0.14	0.83 +/− 0.17	0.95 +/− 0.14	0.53 +/− 0.10
CUS × IMI $F_{(1, 12)} = 2.42$, $p > 0.05$; **CUS** $F_{(1, 12)} = 2.11$, $p > 0.05$; **IMI** $F_{(1, 12)} = 0.24$, $p > 0.05$				
Midbrain	0.30 +/− 0.03	0.45 +/− 0.05	0.60 +/− 0.07*	0.61 +/− 0.11*
CUS × IMI $F_{(1, 16)} = 1.11$, $p > 0.05$; **CUS** $F_{(1, 16)} = 1.36$, $p > 0.05$; **IMI** $F_{(1, 16)} = 10.35$, $p < 0.01$				