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# Chronic stress differentially regulates cannabinoid $CB_1$ receptor binding in distinct hippocampal subfields

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ABSTRACT

Exposure to chronic stress has been found to decrease cannabinoid $CB_1$ receptor expression in the hippocampus; however, the specificity of this phenomenon to specific subfields of the hippocampus has not been characterized. To this extent, male Sprague-Dawley rats were exposed to 21 days of restraint stress (6 h/day), after which autoradiographical analysis of cannabinoid $CB_1$ receptor binding site densities were examined in the CA1, CA3 and dentate gyrus subfields of the hippocampus. Chronic stress was found to produce a significant reduction in cannabinoid $CB_1$ receptor binding in the dentate gyrus, while increasing cannabinoid $CB_1$ receptor binding in the CA3. There was no effect of chronic stress on cannabinoid $CB_1$ receptor binding in the CA1, or two other proximal regions, the retrosplenial cortical gyrus and the laterodorsal thalamus. Given the role of hippocampal cannabinoid $CB_1$ receptor activity in the maintenance of synaptic integrity and neuroplasticity in the hippocampus, these data suggest that changes in cannabinoid $CB_1$ receptor activity following stress may contribute to stress-induced modulation of these processes.



## 1. Introduction

The endocannabinoid system is a neuromodulatory system composed of at least two endogenous ligands, *N*-arachidonylethanolamine (anandamide; AEA) and 2-arachidonylglycerol (2-AG) and at least one receptor, the cannabinoid $CB_1$ receptor, whose expression is predominately presynaptic (Freund et al., 2003). The cannabinoid $CB_1$ receptor is a G protein coupled receptor which binds to $G_{i/o}$ proteins, and is distributed across an array of neuronal populations, such as GABAergic, glutamatergic and monoaminergic neurons (Freund et al., 2003). Activation of the cannabinoid $CB_1$ receptor on axon terminals results in a suppression of neurotransmitter release due to a concomitant inhibition of calcium influx and an amplification of internal potassium conductance (Schlicker and Kathmann, 2001; Freund et al., 2003).

While the cannabinoid $CB_1$ receptor is widely distributed throughout the brain, substantial research has focused on its functional localization within the hippocampus, a region in which the cannabinoid $CB_1$ receptor appears to be an integral player in the regulation of synaptic and structural plasticity (Hashimotodani et al., 2007). In particular, cannabinoid $CB_1$ receptor signalling in the hippocampus is known to contribute to the regulation of neuroplastic phenomenon, such as long-term potentiation (Carlson et al., 2002), depolarization-induced suppression of inhibition (Wilson et al., 2001) and cell proliferation/neurogenesis (Aguado et al., 2005; Jin et al., 2004).

Cannabinoid $CB_1$ receptor expression in the hippocampus is exquisitely sensitive to environmental stress, and both chronic stress and glucocorticoid administration have reliably been found to down-regulate the protein expression and binding site density of the cannabinoid $CB_1$ receptor in the hippocampus (Hill et al., 2005, 2008a,b). These findings become particularly interesting in light of the fact that stress is known to adversely modulate synaptic and structural plasticity within the hippocampus (Gould et al., 1997; Pavlides et al., 2002; McEwen, 2001). However, to date all of the studies examining the effects of stress/glucocorticoids on cannabinoid $CB_1$ receptor binding site densities have employed radioligand binding to membrane fractions generated from whole hippocampi (Hill et al., 2005, 2008a,b), shedding no light on the neuroanatomical specificity of the effects of stress on hippocampal cannabinoid $CB_1$ receptor expression. Given that cannabinoid $CB_1$ receptor signalling within distinct subregions of the hippocampus (CA1, CA3 and dentate gyrus) differentially contributes to synaptic integrity and neuroplasticity, determination of the regional regulation of hippocampal cannabinoid $CB_1$ receptor expression following stress is of importance to understand the functional link between stress, cannabinoid $CB_1$ receptor signalling and neuroplastic phenomena.

## 2. Methods

### 2.1. Animals

Adult male Sprague-Dawley rats were obtained from Charles River Laboratories (Kingston, NY) at 70 days of age. Animals were housed 2–3 per cage (same age cage mates) in clear polycarbonate cages with wood

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chip bedding. All animals were maintained on a 12 h light-dark schedule (lights on at 0800 h) and the temperature was kept at 21 ± 2 °C. All animals had *ad libitum* access to food and water. All procedures were carried out in accordance with the guidelines established by the NIH Guide for the Care and Use of Laboratory Animals.

### 2.2. Stress protocol

Animals were left undisturbed after arrival for one week after delivery. Stressed animals were restrained in wire mesh restrainers, secured at the head and tail ends with large binder clips. Chronic stress was administered for 6 h daily for 21 days from 10:00 to 16:00. Animals were returned to their home cages immediately after termination of the stressor. These animals were sacrificed by decapitation roughly 24 h after the last stress (i.e. between 1300 and 1700 h). Brains were removed and flash frozen on dry ice and then stored at −80°°C until processing. This stress paradigm was employed as it is known to elicit changes in cognitive, emotional and aggressive behaviour, as well as produce morphological changes within the hippocampus (Wood et al., 2003, 2004; Kleen et al., 2006).

### 2.3. Cannabinoid $CB_1$ receptor autoradiography

Autoradiographical analysis of cannabinoid $CB_1$ receptor binding was based on a readily employed protocol that was previously established (Herkenham et al., 1991). Briefly, coronal sections encompassing the dorsal regions of the hippocampus (20 μm thick) were cut in a cryostat and thaw mounted onto gelatine coated slides and dessicated overnight at 0 °C. Slide mounted brain sections were brought to room temperature and incubated for 2.5 h at 37 °C, in a buffer (pH 7.4) containing 50 mM TRIS with 5% bovine serum albumin (fatty-acid free) and 10 nM [$^3$H]-CP,55940 (prepared in the same buffer). An adjacent set of slides was prepared in the identical manner but co-incubated in the presence of 10 μM non-radiolabelled CP-55,940 to determine non-specific binding (which was then subtracted from the total binding). Following this incubation, slides were washed in 50 mM TRIS buffer (pH 7.4) with 1% bovine serum albumin (fatty-acid free) for 4 h (2×2 h washes) at 0 °C, dipped in ice cold distilled water and dried under a stream of cool air.

Autoradiograms were generated by apposing the labelled tissues together with autoradiographic standards ([$^3$H] micro-scales, Amersham), to tritium sensitive film ([$^3$H]-Hyperfilm, Amersham) for a period of two weeks. Autoradiograms were developed (D-19, Kodak) for 4 min at 20 °C, and the films were analyzed and quantified in a computer-assisted video densitometer using the curve generated from [$^3$H]-standards. In addition to analysis of cannabinoid $CB_1$ receptor binding site densities in hippocampal subfields, the effects of chronic stress on cannabinoid $CB_1$ receptor binding site densities were also examined in two proximal regions, the retrosplenial cortical gyrus and laterodorsal thalamus, as control regions to determine if the effects of stress were specific to the hippocampus.

### 2.4. Statistics

Data regarding the effects of stress on cannabinoid $CB_1$ receptor binding in hippocampal subfields, retrosplenial cortical gyrus and laterodorsal thalamus were analyzed using an independent *t*-test. Significance was established at a *P* value less than 0.05.

## 3. Results

Exposure to 21 days of restraint stress was found to significantly decrease cannabinoid $CB_1$ receptor binding site densities within the dentate gyrus [$t$ (14) = 2.72, $P$<0.02]. Alternately, 21 days of restraint stress was found to increase cannabinoid $CB_1$ receptor binding site densities within the CA3 region of the hippocampus [$t$ (14) = 2.19, $P$<0.05]. There was no effect of 21 days of restraint stress on cannabinoid $CB_1$ receptor binding in the CA1 region of the hippocampus [$t$ (14) =

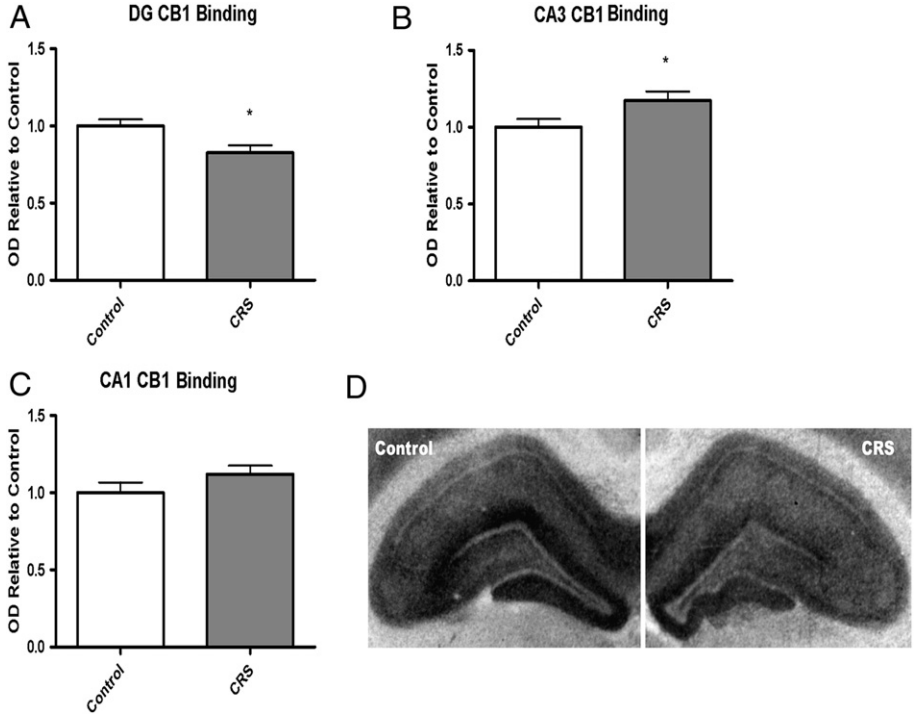


**Fig. 1.** The effect of chronic restraint stress (CRS; 21 days at 6 h/day) on cannabinoid $CB_1$ receptor binding in the (A) dentate gyrus, (B) CA3 and (C) CA1 subfields of the hippocampus. Values are denoted as mean relative optical density (R.O.D.) ± S.E.M. * denotes significant differences relative to all other treatment conditions ($n$ = 7–8/condition). (D) Representative autoradiograms of the effects of chronic restraint stress (CRS) on cannabinoid $CB_1$ receptor binding throughout the hippocampus.

1.34, $P>0.05$]. Similarly, there was no effect of chronic stress on cannabinoid $CB_1$ receptor binding in either the laterodorsal thalamus [t (14) = 1.21, $P>0.05$; control = 0.035 ± 0.004 vs. chronic stress = 0.029 ± 0.004] or the retrosplenial cortical gyrus [$t$ (14) = 0.59, $P>0.05$; control = 0.036 ± 0.003 vs. chronic stress = 0.038 ± 0.003]. Data regarding the effects of chronic stress on cannabinoid $CB_1$ receptor binding on hippocampal subfields, as well as a representative autoradiogram of cannabinoid $CB_1$ receptor binding, can be seen in Fig. 1.

## 4. Discussion

Within this study, exposure to chronic stress was found to significantly down-regulate cannabinoid $CB_1$ receptor binding in the dentate gyrus of the hippocampus, but increase cannabinoid $CB_1$ receptor density in the CA3; there was no effect of chronic stress on cannabinoid $CB_1$ receptor binding in the CA1. Within this coronal plane of the brain, these effects were specific to hippocampal subfields as there was no effect of chronic stress on cannabinoid $CB_1$ receptor binding in two proximal regions, the retrosplenial cortical gyrus and the laterodorsal thalamus. This data does not imply that chronic stress does not alter cannabinoid $CB_1$ receptor binding parameters in other brain regions involved in stress regulation or emotionality (such as the prefrontal cortex, which is known to exhibit an upregulation of the cannabinoid $CB_1$ receptor in response to stress; Hill et al., 2008b) but suggests that the effects of stress are limited to specific brain regions, and subregions therein.

Previous research has found that cannabinoid $CB_1$ receptor expression and binding within the hippocampus (when examined in tissue homogenates generated from the entire hippocampus) is decreased by chronic stress (Hill et al., 2005, 2008b); these findings suggest that this downregulation is seemingly mediated by a reduction of cannabinoid $CB_1$ receptor binding site densities in the dentate gyrus. More so, this reduction is presumably driven by the elevated secretion of adrenal steroids that occurs during chronic stress, as prolonged administration of the glucocorticoid hormone corticosterone evokes a comparable reduction in cannabinoid $CB_1$ receptor binding site densities and protein expression (Hill et al., 2008a).

Cannabinoid $CB_1$ receptor signalling in the dentate gyrus is known to be important for the regulation of synaptic transmission; accordingly, the current data suggest that reductions in cannabinoid $CB_1$ receptor levels in this region may relate to stress-induced alterations in excitatory neurotransmission. For example, stress/glucocorticoids can worsen neurological insults produced by excitotoxic damage, and diseases characterized by hyper-excitable hippocampal circuits (such as epilepsy) are exacerbated by stress and glucocorticoids (Conrad et al., 2004; DeVries et al., 2001; McEwen, 2001). Electrophysiological work has revealed that cannabinoid $CB_1$ receptors gate excitatory neurotransmission within the dentate gyrus between mossy fiber cell-dentate granule synapses and that impairments in cannabinoid $CB_1$ receptor signalling at this junction can result in hyperexcitability of this circuit (Monory et al., 2006; Chiu and Castillo, 2008), a phenomenon which is characteristic of epileptiform disorders (Ratzliff et al., 2002). More so, local viral deletion of cannabinoid $CB_1$ receptors in the dentate gyrus dramatically worsens kainic acid-induced seizures (Monory et al., 2006), and human epileptics have been found to exhibit profound reductions in cannabinoid $CB_1$ receptor expression in the dentate gyrus (Ludanyi et al., 2008). Taken with the current data set, it seems plausible that a stress-induced downregulation of cannabinoid $CB_1$ receptor expression within the dentate gyrus may enhance vulnerability of this circuit to hyperexcitability and represent a mechanism by which stress can exacerbate neurological conditions, such as epilepsy.

In line with this hypothesis, it is possible that the up-regulation of cannabinoid $CB_1$ receptor binding in the CA3 may represent a compensatory response. Specifically, if reductions in cannabinoid $CB_1$ receptor activity in the dentate gyrus result in an increased excitability of the dentate gyrus-mossy fiber pathway, this would presumably result in an increase in excitatory neurotransmission in the terminal field of this pathway in the CA3 region. Consistent with this, chronic stress does result in elevated excitatory amino acid neurotransmission in the CA3 region of the hippocampus (McEwen, 2001). Similar to the dentate gyrus, activation of cannabinoid $CB_1$ receptors within the CA3 region of the hippocampus reduce glutamatergic transmission, as well as dampen epileptiform neuronal activity (Ameri and Simmet, 2000). Accordingly, an upregulation of cannabinoid $CB_1$ receptors in the CA3 could function to attenuate the increased excitability originating from the dentate gyrus-mossy fiber pathway, in an attempt to normalize neuronal activity throughout the hippocampal circuit.

Collectively, these data demonstrate that exposure to chronic stress evokes subfield specific regulation of cannabinoid $CB_1$ receptor binding throughout the hippocampus. These changes in turn, may relate to alterations in neuronal excitability and synaptic plasticity that occur throughout the hippocampal circuit following stress.

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