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Brain regional differences in CB1 receptor adaptation and regulation of transcription

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Abstract

Cannabinoid CB_1 receptors (CB_1Rs) are expressed throughout the brain and mediate the central effects of cannabinoids, including Δ^9-tetrahydrocannabinol (THC), the main psychoactive constituent of marijuana. Repeated THC administration produces tolerance to cannabinoid-mediated effects, although the magnitude of tolerance varies by effect. Consistent with this observation, CB_1R desensitization and downregulation, as well induction of immediate early genes (IEGs), varies by brain region. Zif268 and c-Fos are induced in the forebrain after acute THC administration. Phosphorylation of the cAMP response-element binding protein (CREB) is increased in a region-specific manner after THC administration. Results differ between acute versus repeated THC injection, and suggest that tolerance to IEG activation might develop in some regions. Repeated THC treatment produces CB_1R desensitization and downregulation in the brain, although less adaption occurs in the striatum as compared to regions such as the hippocampus. Repeated THC treatment also induces expression of ΔFosB, a very stable isoform of FosB, in the striatum. Transgenic expression of ΔFosB in the striatum enhances the rewarding effects of several drugs, but its role in THC-mediated effects is not known. The inverse regional relationship between CB_1R desensitization and ΔFosB induction suggests that these adaptations might inhibit each other, although this possibility has not been investigated. The differential regional expression of individual IEGs by acute or repeated THC administration suggests that regulation of target genes and effects on CB_1R signaling will contribute to the behavioral effects of THC.

Keywords

immediate early genes; cannabinoid receptor; G-protein; ΔFosB; CREB; THC

Introduction

Cannabinoid type 1 receptors (CB_1Rs) are potential therapeutic targets for numerous disorders, but also mediate the psychoactive and motor and memory-impairing effects of cannabinoids, which limits their clinical use. The psychoactive effects of Δ^9-tetrahydrocanabinol (THC), the main psychoactive constituent of marijuana, also contribute to its popularity as an illicit drug. Repeated marijuana use can produce tolerance and withdrawal symptoms, which are included in the DSMIV criteria for cannabis use disorder

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(American Psychiatric Association, 2000). Understanding the molecular mechanisms that underlie these cannabinoid properties is critical to developing strategies to overcome these adverse effects. Studies have shown that tolerance to repeated cannabinoid agonist administration occurs concurrently with CB_1R desensitization (attenuated receptor-mediated G-protein and effector activity) and downregulation (loss of receptors). Studies from our laboratory and others have revealed that CB_1R desensitization and downregulation vary by brain region in rodents treated with THC or synthetic cannabinoids (Sim-Selley, 2003). Similar regional differences in CB_1R downregulation occur in the human brain (Villares, 2007, Hirvonen et al., 2011). CB_1R desensitization and downregulation recover within days to weeks following cessation of treatment (Sim-Selley et al., 2006, Hirvonen et al., 2011), suggesting that long-lasting neurobiological changes produced by cannabinoids are mediated by additional mechanisms. Immediate Early Genes (IEGS) provide candidate mechanisms to regulate both short and longer-term adaptations to cannabinoids. IEGs are transcription factors that can be constitutively expressed or induced by stimuli to regulate the expression of target genes. Inducible IEGs, including zif268 (also called krox24 or egr1) and the Fos (c-Fos, FosB, fos-related antigen 1 (Fra-1), Fra-2 and ΔFosB) and Jun (c-Jun, JunB and junD) families of transcription factors can be regulated by cannabinoids. Cannabinoids also regulate cAMP response element binding protein (CREB), which is constitutively expressed and its binding to DNA is regulated by phosphorylation by upstream kinases. This review will discuss cannabinoid-mediated regulation of these transcription factors in the brain and consider the possible functional consequences.

CNS expression of CB_1Rs and IEGs

Co-distribution of CB_1Rs and IEGs in the brain provides potential interactions that could influence a variety of *in vivo* responses. CB_1Rs are widely expressed in the brain, with high density in the prefrontal cortex, globus pallidus, substantia nigra, hippocampus, striatum (caudate-putamen and nucleus accumbens) and molecular layer of the cerebellum. Lower expression occurs in the hypothalamus, periaqueductal gray and basolateral amygdala. This expression profile corresponds with acute cannabinoid-mediated effects, including antinociception, catalepsy, hypolocomotion, hypothermia and memory impairment (Howlett et al., 2002). Inducible transcription factors are basally expressed in the brain and exhibit species-specific regional differences in basal expression (Herdegen and Leah, 1998). Basal IEG and CB_1R expression have not been directly compared, but can be compared indirectly using the BrainStars (B*) database of DNA-microarray data in mouse brain (Kasukawa et al., 2011). Comparisons of CB_1R with zif268, CREB, c-Fos, and FosB show that mRNA for these proteins are expressed in all regions examined. A brain-region dependent correlation between CB_1R and zif268 mRNA expression was found [$r(100) = 0.35$, $p < 0.001$]. Cannabinoids induce IEGs in unique regional patterns that provide for both anatomical and IEG-specific interactions, as discussed in subsequent sections.

Cannabinoid-Regulated Immediate Early Genes

The effect of cannabinoid administration on specific IEGs is discussed in the following sections. As shown in Table 1, acute versus repeated cannabinoid administration can regulate IEGs differently. As reported for other measures, differences in the drug and dose administered, timing of administration and species examined can produce different results between laboratories. The time between cannabinoid administration and tissue collection can also influence results, because many IEGs are only transiently induced after treatment. The method of IEG analysis also influences results. Immunocytochemistry and in situ hybridization provide anatomical resolution, but these measurements are considered semi-quantitative. Measurement of proteins or mRNA in membranes of dissected regions can provide more accurate quantification, but limits anatomical resolution. Studies have often

focused on regions that contain high levels of CB_1Rs and are readily dissected (e.g. striatum, hippocampus, cerebellum), but additional regions also contribute to physiological and behavioral effects of cannabinoids.

The transcriptional activity of individual IEGs should also be considered in interpreting results. Transcriptional repressors also exist, such as cAMP response-element modulator (CREM), which reduces CREB transcription, and Fos-related antigen 1 (Fra1), which reduces the transcriptional ability of AP-1 complexes (Foulkes and Sassone-Corsi, 1992, Yoshioka et al., 1995). IEGs can also induce or repress the expression of other IEGs. For example, CREB can induce *c-fos* mRNA (Sheng et al., 1991), whereas ΔFosB, a truncated splice variant of FosB, can repress *c-fos* mRNA expression through epigenetic regulation by recruitment of histone deacetylase 1 (HDAC1) (Renthal et al., 2008). Co-regulation adds to the complexity of understanding interactions among IEGs and provides multiple points for interactions between these signaling pathways.

Zif268

Expression of zif268 in the brain has been implicated in the regulation of neural plasticity, the proteosome complex and long term potentiation/memory formation (James et al., 2006). Acute cannabinoid administration enhances zif268 expression, whereas repeated treatment reduces expression. Mailleux et al. (1994) reported that *zif268* mRNA increased in the cingulate cortex, fronto-parietal cortex and caudate-putamen of rats 20 minutes after acute THC (5 mg/kg) injection. Separate studies in the caudate-putamen showed that zif268-immunoreactive (-ir) cells were restricted to striosomes when assessed 2 hours after injection of CP55,940 (2.5 mg/kg) (Glass and Dragunow, 1995). Striosome-specific IEG expression has also been reported after administration of cocaine or amphetamine (Moratalla et al., 1992, Capper-Loup et al., 2002). This finding could be relevant for motivated behavior because rodents more reliably lever press for electrical stimulation in striatal striosomes compared to the matrix (White and Hiroi, 1998).

Studies in the hippocampus showed that acute THC (1 mg/kg) increased *zif268* mRNA in CA1 and CA3, but not dentate gyrus, in CD1 mice (Derkinderen et al., 2003). Expression of zif268 in the hippocampus could contribute to the memory impairing effects of THC. This question was addressed by comparing the effects of THC in the Morris water task with changes in numbers of zif268-ir cells in various brain regions (Boucher et al., 2009). Mice (C57Bl6) were treated with THC (1 mg/kg) or vehicle for 13 days, then tested each day for 11 days with THC (1 mg/kg) or vehicle in the Morris water task, and brains were collected. A separate group of mice did not receive pretreatment but were similarly tested for 11 days in the Morris water task. The number of zif268-ir cells was increased for all mice tested in the Morris water task, including vehicle-vehicle-treated, in hippocampus CA1 and CA3, prefrontal cortex and caudate-putamen when compared to home cage mice that underwent no manipulations. This indicates that learning the task, regardless of drug treatment, increased zif268 expression in these regions. The number of zif268-ir cells in CA3, prefrontal cortex and caudate-putamen was decreased in mice treated with THC during the 11 days of testing when compared to mice that received vehicle during testing. This suggests that the combination of neuronal activity with cannabinoid treatment differentially affected IEG expression. Zif268 knockout mice have also been evaluated (Tzavara et al., 2001). No genotype-specific differences in cannabinoid analgesia or withdrawal were reported, but these results do not preclude a role for zif268 in other measures.

CREB

Several drugs of abuse increase CREB activity, measured as CREB phosphorylation (pCREB) or total CREB bound to DNA (Nestler, 2004). Initial studies showed no changes

in CREB bound to DNA in the caudate-putamen or cerebellum of rats that received THC (5–40 mg/kg b.i.d) for 5 days with brain collection 21 days after the last injection (Rubino et al., 2003). Subsequent studies using acute THC (15 mg/kg) administration found increased pCREB levels in the caudate-putamen, hippocampus and cerebellum, but not prefrontal cortex, of rats when measured 30 minutes following injection (Rubino et al., 2004). A different regional pattern emerged following repeated THC administration (15 mg/kg, b.i.d., 6.5 days), whereby pCREB was only increased in the prefrontal cortex of THC-treated rats. This finding could indicate that tolerance developed to THC-induced activation of CREB in the other regions, and highlights the time-dependent nature of IEG expression. A separate study examined CREB in the granule cell layer of the rat cerebellum. CB_1Rs are expressed on granule cell axons in the molecular layer of the cerebellum. Results showed an increase in pCREB-ir cells in the granule cell layer following acute administration of 5 or 10 mg/kg THC, whereas repeated THC (10 mg/kg q.d., 4 weeks) administration produced a decrease in pCREB-ir that persisted for 3 weeks (Casu et al., 2005). This finding highlights the temporal nature of CREB activation, and suggests that alterations in CREB activity can persist after cessation of drug treatment.

Measurement of CREB in the hippocampus following repeated THC administration has provided varying results. In one study, CREB and pCREB were decreased in the hippocampus in C57BL6 mice administered THC (10 mg/kg q.d.) for 7 days with levels assessed 24 hours after the last administration (Fan et al., 2010). Another group reported that repeated THC (10 mg/kg, b.i.d.) administration in rats for 4.5 days increased pCREB when tested 30 minutes after the final administration (Rubino et al., 2006). Differences in results could reflect methodological differences between the studies, most notably the survival time following final THC injection.

Brain-derived neurotrophic factor (BDNF) is regulated at the transcriptional level by CREB (McClung and Nestler, 2003) and has been measured following THC treatment. Both acute (Derkinderen et al., 2003) and repeated (Rubino et al., 2006) THC administration increased BDNF in the hippocampus. A recent study showed that intracerebroventricular injection of BDNF reduced HU210-stimulated CB_1R activity at GABAergic striatal synapses via regulation of cholesterol metabolism and lipid rafts (De Chiara et al., 2010). Thus, IEG-regulated BDNF expression could regulate the endocannabinoid system.

Possible consequences of CB_1R-regulated CREB activation are suggested by studies utilizing THC infusion into specific brain regions, with subsequent measurement of pCREB and anxiolytic responses in rodents (Rubino et al., 2008). Infusions of THC (1 μg–10 μg) into the prefrontal cortex or ventral hippocampus produced anxiolysis and increased pCREB-ir, whereas infusion into the basolateral amygdala produced anxiogenic effects and decreased pCREB-ir levels. These findings show that both regulation of CREB activity and subsequent functional effects are region-dependent.

c-Fos

Fos (c-Fos, FosB, fos-related antigen 1 (Fra-1), Fra-2 and ΔFosB) and Jun (c-Jun, JunB and junD) families of transcription factors form AP-1 complexes that bind to AP-1 consensus sites on target genes. Mailleux et al. (1994) showed that c-Fos-ir and c-Jun-ir cells increased in the cingulate cortex when measured 20 minutes after THC (5 mg/kg) injection, whereas only c-Fos-ir cells increased in the fronto-parietal cortex and caudate-putamen. Subsequent studies showed an increase in c-Fos-ir cells in the caudate-putamen and nucleus accumbens of rats when measured 2 hours after THC injection (10 mg/kg) (Miyamoto et al., 1996). In this same study, pretreatment with a dopamine D_1 receptor (D_1R) antagonist (SCH-23390, 0.32 mg/kg), but not a D_2 receptor (D_2R) antagonist ((-)-sulpiride,100 mg/kg, i.p.),

significantly attenuated c-Fos induction in these regions, suggesting that c-Fos induction was due to CB_1R-mediated dopamine release and not through direct CB_1R signaling. The same group measured c-Fos-ir following repeated THC administration (10 mg/kg, q.d., 4 days) at 2 hours after final injection and compared the results to acute induction (Miyamoto et al., 1997). Repeated THC administration induced fewer c-Fos-ir cells as compared to acute administration, suggesting the development of tolerance. A similar study also suggested that tolerance developed to the induction of c-Fos in the prefrontal cortex and cerebellum following repeated, but not acute, THC (15 mg/kg) administration (Rubino et al., 2004). The mechanism underlying this effect is not known, but could involve CB_1R desensitization/downregulation or epigenetic changes through ΔFosB regulation of HDAC1 (Renthal et al., 2008).

Comparison of *c-fos* mRNA expression following acute administration of THC (25 mg/kg), morphine (10 mg/kg) or cocaine (50 mg/kg) showed regionally distinct patterns of c-Fos induction (Erdtmann-Vourliotis et al., 1999). THC induced *c-fos* mRNA in the lateral septum, paraventricular nucleus, caudate-putamen and nucleus accumbens, which was similar to lysergic acid diethylamide (LSD) (1 mg/kg) and 3,4-methylenedioxymethamphetamine (MDMA) (6 mg/kg). THC also increased *c-fos* mRNA in the mediodorsal thalamus, whereas LSD and MDMA induced *c-fos* mRNA in cortical layers that were not observed after THC. Expression of *c-fos* mRNA following cocaine treatment was restricted to the caudate-putamen, whereas morphine induced expression only in the lateral septum and paraventricular nucleus. Another group found a somewhat different regional expression of *c-fos* mRNA following acute injections of THC (5 mg/kg), morphine (20 mg/kg) and cocaine (20 mg/kg) (Marie-Claire et al., 2003). In this study, *c-fos* mRNA was increased in the prefrontal cortex, nucleus accumbens, caudate-putamen and hippocampus after both THC and cocaine injections, whereas morphine increased *c-fos* mRNA only in the caudate-putamen and hippocampus. There were several differences between these studies, including the use of in situ hybridization (Erdtmann-Vourliotis et al., 1999) versus real time PCR (Marie-Claire et al., 2003) to measure *c-fos*. Nevertheless, results show drug- and region-specific induction of *c-fos* mRNA.

FosB and ΔFosB

Fewer studies have assessed FosB and its truncated isoforms (ΔFosB, Fra-1 and Fra-2) following cannabinoid treatment. Fos antigens are generally induced rapidly and transiently after acute drug administration (e.g. c-Fos). However, ΔFosB, a C-terminally truncated splice variant of FosB, is stable and accumulates with repeated induction over time (e.g. during repeated drug treatment), and can be detected in neurons for several weeks after cessation of drug treatment (Chen et al., 1997, Perrotti et al., 2005, Ulery et al., 2006). ΔFosB could therefore be important in regulating the long-term effects of repeated cannabinoid administration. THC administration increased Fos proteins (c-Fos, FosB, Fra-1 and Fra-2) and AP1 DNA binding in the nucleus accumbens when measured one hour following administration of 10 or 15, but not 5, mg/kg of THC in rats (Porcella et al., 1998). AP-1 binding in the cingulate cortex and caudate-putamen was increased only after the highest dose of THC. In the cingulate cortex, this occurred in conjunction with increased c-Fos FosB, Fra-1 and Fra-2, whereas in the caudate-putamen, only c-Fos and FosB were significantly induced. ΔFosB was not significantly induced in any region examined, which is consistent with its low level of induction after a single drug injection. Induction of c-Fos, FosB, Fra-1 and Fra-2 was CB_1R-mediated because it was blocked by pretreatment with the antagonist SR141716A (Rimonabant) (Porcella et al., 1998). Regional assessment of FosB following acute and repeated THC administration showed increased FosB in prefrontal cortex and hippocampus only after repeated THC administration (Rubino et al., 2004).

We have compared the ability of several drugs of abuse, including THC, to induce ΔFosB by using immunohistochemistry (Perrotti et al., 2008). FosB/ΔFosB-ir cells were counted 24 hours after the last drug injection, a time point that favors ΔFosB because other FosB isoforms are typically degraded by this time. Repeated THC administration significantly increased the number of FosB/ΔFosB-ir cells in the nucleus accumbens core with trends toward increases in the nucleus accumbens shell and caudate-putamen. Increases in these three regions were also produced by alcohol, morphine and cocaine, suggesting that a common anatomical substrate might underlie ΔFosB-mediated neuroadaptation. Investigation of ΔFosB is facilitated by transgenic models developed by Nestler and colleagues, in which the tetracycline gene regulation system is used to express ΔFosB or Δc-Jun, a dominant negative inhibitor of AP1-mediated transcription, in a regionally and temporally specific manner in brain (Chen et al., 1998, Peakman et al., 2003). Mice in which ΔFosB is expressed in D_1R/dynorphin-positive striatal medium spiny neurons, show enhanced reward for several drugs of abuse, including cocaine and morphine, as well as natural rewards (Nestler, 2008). We recently used this model to determine that expression of ΔFosB enhanced signaling by mu (MOR) and kappa (KOR) opioid receptors, but did not alter CB_1R signaling, in the nucleus accumbens (Sim-Selley et al., 2011). Enhanced KOR signaling could be a response to the reduced dynorphin that has been seen in ΔFosB-expressing mice (Zachariou et al., 2006). It is possible that CB_1R signaling changes in a sub-population of neurons, such as the nucleus accumbens core (Perrotti et al., 2008), so that alterations were masked in whole nucleus accumbens membrane preparation. Moreover, this line of bitransgenic mice expresses ΔFosB only in D_1R/dynorphin medium spiny neurons, but CB_1R are expressed in both D_1R/dynorphin and D_2R/enkephalin positive striatal neurons (Hohmann and Herkenham, 2000), as well as on terminals of cortical afferents (Robbe et al., 2001). Alternatively, cannabinoid-induced ΔFosB could indirectly affect CB_1R function. For example, repeated administration of CP55,940 increased MOR-mediated signaling in the nucleus accumbens (Vigano et al., 2005), similar to our findings in ΔFosB-expressing mice (Sim-Selley et al., 2011). The effect of ΔFosB on THC-mediated behaviors is not known. Our results showed that ΔFosB induced alterations in MOR and KOR/dynorphin in the striatum (Zachariou et al., 2006, Sim-Selley et al., 2011). MOR null mice do not exhibit THC place preference, whereas KOR deletion reduces THC place aversion and reveals THC place preference (Ghozland et al., 2002). Similarly, prodynorphin null mice do not exhibit THC conditioned place aversion like wild-type mice (Zimmer et al., 2001) and prodynorphin null mice show a leftward shift in the dose-intake curve of WIN55,212-2 self-administration (Mendizabal et al., 2006). These findings suggest that ΔFosB induction by repeated THC administration could influence the pharmacological effects of cannabinoids via modulation of endogenous opioid systems.

We recently compared ΔFosB induction and CB_1R desensitization and downregulation in the same brains following THC (10 mg/kg b.i.d.) administration for 14 days (Lazenka et al., 2011). CB_1R desensitization and downregulation were found in prefrontal cortex, hippocampus, lateral amygdala and basomedial amygdala. ΔFosB was significantly induced in the prefrontal cortex, nucleus accumbens, caudate-putamen, lateral amygdala and cerebellum, with no change in the hippocampus or basomedial amygdala. Analysis revealed a significant inverse regional correlation between ΔFosB induction and CB_1R desensitization, where greater induction of ΔFosB correlated with lower magnitude of CB_1R desensitization in a brain-region dependent manner. These findings suggest that ΔFosB might inhibit CB_1R desensitization in regions such as the striatum and/or that CB_1R desensitization could inhibit ΔFosB induction in regions including the hippocampus. Potential interactions between these CB_1R-mediated adaptations have not yet been investigated at a mechanistic level, but such studies could reveal novel interactions between IEG induction and receptor adaptation. Future studies using genetic overexpression, deletion

or dominant negative inhibition of ΔFosB will be important to establish a link between these adaptive mechanisms.

CB_1R Desensitization and Downregulation

Studies have shown that CB_1Rs in the caudate-putamen and its projection areas (globus pallidus and substantia nigra) show the least magnitude of CB_1R desensitization and downregulation, whereas CB_1Rs in the hippocampus exhibit the greatest magnitude of desensitization and downregulation in response to repeated THC administration (Sim-Selley, 2003). Similarly, CB_1R adaptations in the striatum develop more slowly and recover more quickly than in regions such as the hippocampus (Breivogel et al., 1999, Sim-Selley et al., 2006). Slower recovery of hippocampal CB_1Rs has also been reported in human marijuana users (Hirvonen et al., 2011). The potential relevance of these findings is supported by human studies that showed greater tolerance to the memory impairing and anxiogenic effects of THC than to its psychoactive and motor effects (Haney et al., 2004, D'Souza et al., 2008). The role of IEGs in CB_1R desensitization and downregulation, and possible region-specific adaptations, are not known. A recent study in a mouse model of Huntington's disease suggests that the repressor element 1 silence transcription factor (REST) can regulate transcription of CB_1Rs (Blazquez et al., 2011), and it is possible that cannabinoid-induced IEGs could regulate CB_1R expression.

ΔFosB reduces BDNF expression (McClung and Nestler, 2003) and might regulate endocannabinoids as discussed for CREB. Induction of ΔFosB in the striatum could reduce BDNF expression and thereby inhibit negative regulation of CB_1R function. Other studies have suggested that inhibition of extracellular signal-regulated kinase (ERK) phosphorylation regulates CB_1R desensitization in the caudate-putamen and cerebellum (Rubino et al., 2005). Inhibition of ERK also blocks induction of FosB and BDNF, as well as phosphorylation of CREB, in the prefrontal cortex and hippocampus following repeated THC administration (Rubino et al., 2006). It is difficult to predict whether ERK might regulate CB_1R adaptations directly or indirectly through ERK-mediated induction of IEGs (Herdegen and Leah, 1998). Studies with protein kinase A (PKA) inhibitors have suggested a role for this kinase in facilitating tolerance to the antinociceptive and hypolocomotor, but not the hypothermic, effects of THC (Lee et al., 2003, Bass et al., 2004, Martin et al., 2004). It is not yet clear whether this effect of PKA inhibition occurs via inhibition of CB_1R desensitization and dowregulation. Cyclin-dependent kinase 5 (CDK5) is a target of ΔFosB (McClung and Nestler, 2003) that could also regulate these kinases by regulating the dopamine- and cAMP regulated phosphoprotein, *M*r 32,000 DARPP-32 (DARPP-32). Phosphorylation of DARPP-32 at threonine 75 can reduce both ERK and PKA activity, and CDK5 phosphorylates DARPP-32 at this site (Bibb et al., 1999, Valjent et al., 2005). Acute cannabinoid administration increases DARPP-32 phosphorylation at threonine 34; however, it has not yet been determined if repeated THC administration increases expression of CDK5 or regulates DARPP-32 phosphorylation(Borgkvist and Fisone, 2007).

The expression of transcription factors and epigenetic changes might contribute to the abuse potential of cannabinoids, because CREB modulates the development of dependence and ΔFosB enhances the rewarding and motivational effects of drugs of abuse (Nestler, 2004, Robison and Nestler, 2011). Moreover, changes in CB_1R-mediated signaling could modulate the effects of other drugs of abuse, because CB_1R antagonist administration or receptor deletion reduces the rewarding effects of several drugs of abuse, and the endocannabinoid system is involved in reinstatement of drug seeking behavior (Carai et al., 2005, Valverde et al., 2005, Maldonado et al., 2006, Wiskerke et al., 2008). The identification of cannabinoid-regulated IEGs and their target genes is an important step that could provide new targets for treatment of drug abuse.

Conclusions

Acute administration of THC induces IEGs, including zif268, pCREB and c-Fos, in a brain region-dependent manner, with most studies reporting induction in the striatum, hippocampus and cortex. Repeated THC administration appears to produce less induction of CREB and zif268 in certain regions, suggesting the possible development of tolerance to this effect. The caudate-putamen and nucleus accumbens are of particular interest for their role in motivation and motor behaviors. CB_1Rs in these regions exhibit less desensitization and downregulation than other brain regions, which is consistent with reports that humans exhibit less tolerance to THC-mediated subjective and motor effects. The mechanisms underlying these observations are not defined, but findings suggest that induction of IEGs might be involved. An inverse regional correlation was found between CB_1R desensitization and ΔFosB expression, suggesting that ΔFosB might inhibit CB_1R adaptation and/or CB_1R desensitization could inhibit ΔFosB induction. ΔFosB is especially interesting because its expression in D_1R/dynorphin striatal neurons enhances the rewarding effects of drugs of abuse, although its role in THC-mediated motivational effects is not known. Certain gene targets of ΔFosB have been identified, some of which might regulate THC-mediated effects. For example, we showed that transgenic overexpression of ΔFosB enhanced MOR and KOR signaling, and previous studies suggest a role for opioid systems in motivational effects of THC. BDNF is regulated by ΔFosB and CREB and has been implicated in endocannabinoid regulation in the striatum. Several downstream kinases that regulate repeated THC-mediated effects are targets of IEGs, either directly or indirectly, and contribute to signaling pathways that could regulate CB_1Rs. However, the role of IEGs in CB_1R adaptation has not been investigated. It is also possible that CB_1R desensitization and downregulation modulate CB_1R-mediated IEG induction, but this possibility has not been examined. Future studies are likely to focus on signaling pathways that link IEGs and CB_1R desensitization/downregulation and determine the functional consequences of these adaptations.

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Table 1

Summary of brain-region dependent changes in IEG expression following acute or repeated THC administration.

Transcription factor	Treatment (time after last injection)	Increase in Brain Region	Decrease in Brain Region	Measure	Reference
Zif268					
Acute	5 mg/kg THC (20 minutes)	cingulate cortex, fronto-parietal and caudate-putamen		mRNA immunohistochemistry	Mailleux et al.,1994
Acute	2.5 mg/kg CP55,940 (2 hours)	striosome of caudate-putamen		mRNA immunohistochemistry	Glass and Dragunow, 1995
Acute	1 mg/ml THC (60 minutes)	hippocampus CA1 and CA3		mRNA immunohistochemistry	Derkinderen et al., 2003
Repeated	1 mg/kg THC q.d. for 11 days (90 minutes after last probe trial)		prefrontal cortex, caudate-putamen and CA3 (compared to vehicle controls)	protein immunohistochemistry	Boucher et al., 2009
CREB					
Acute	15 mg/kg THC (30 minutes)	caudate-putamen, hippocampus and cerebellum		pCREB protein bound to DNA ELISA	Rubino et al., 2004
Acute	5 or 10 mg/kg THC (90 minutes)	Cerebellum		pCREB protein immunohistochemistry	Casu et al., 2005
Acute	1 μg, 5 μg or 10 μg THC microinjection (immediately after elevated plus maze)	prefrontal cortex (10 μg) and ventral hippocampus (5 μg)	basolateral amygdala (1 μg)	(pCREB) Immunoblot	Rubino 2008
Repeated	15 mg/kg THC b.i.d. for 6.5 days (30 minute)	prefrontal cortex		pCREB protein bound to DNA ELISA	Rubino et al., 2004
Repeated	10 mg/kg THC q.d. for 4 weeks (24 hours or 3 weeks)		cerebellum	pCREB protein immunohistochemistry	Casu et al., 2005
Repeated	10 mg/kg THC 4.5 days (30 minutes)	hippocampus		pCREB protein bound to DNA ELISA	Rubino et al., 2006
Repeated	10 mg/kg THC 7 days (24 hours)		hippocampus	pCREB and total CREB protein Immunoblot	Fan et al., 2010
c-Fos					

Transcription factor	Treatment (time after last injection)	Increase in Brain Region	Decrease in Brain Region	Measure	Reference
Acute	5 mg/kg THC (20 minutes)	cingulate cortex, fronto-parietal and caudate-putamen		mRNA immunohistochemistry	Mailleux et al., 1994
Acute	10 mg/kg THC (2 hours)	caudate-putamen and nucleus accumbens		protein immunohistochemistry	Miyamoto et al., 1996
Acute	25 mg/kg THC (1 hour)	lateral septum, paraventricular nucleus, caudate-putamen, nucleus accumbens and mediodorsal thalamus	prefrontal cortex and cerebellum	mRNA immunohistochemistry	Erdtmann-Vourliotis et al., 1999
Acute	5 mg/kg THC (1 hour)	prefrontal cortex, nucleus accumbens, caudate-putamen and hippocampus		mRNA RT-PCR	(Marie-Claire et al., 2003
Repeated	15 mg/kg THC b.i.d. for 6.5 days (30 minute)	prefrontal cortex and cerebellum		c-Fos protein bound to DNA ELISA	Rubino et al., 2004
FosB					
Acute	10 mg/kg and 15 mg/kg THC (1 hour)	nucleus accumbens		FosB, Fra-1 and Fra-2 protein immunoblot	Porcella et al., 1998
Acute	15 mg/kg THC (1 hour)	caudate-putamen		FosB protein Immunoblot	Porcella et al., 1998
Acute	15 mg/kg THC (1 hour)	cingulate cortex		FosB, Fra-1 and Fra-2 protein immunoblot	Porcella et al., 1998
Repeated	15 mg/kg THC b.i.d. for 6.5 days (30 minute)	prefrontal cortex and hippocampus		FosB protein bound to DNA ELISA	Rubino et al., 2004
ΔFosB					
Repeated	10–150 mg/kg q.d. for 14.5 days (24 hours)	nucleus accumbens core		protein immunohistochemistry	Perrotti et al. 2008
Repeated	10 mg/kg q.d. for 13.5 days (24 hours)	prefrontal cortex, caudate-putamen, nucleus accumbens and cerebellum		protein immunoblot	Lazenka et al. 2011