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# Endocannabinoid signaling in reward and addiction

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## Abstract

Brain endocannabinoid signaling influences the motivation for natural rewards (such as palatable food, sexual activity and social interaction) and modulates the rewarding effects of addictive drugs. Pathological forms of natural and drug-induced reward are associated with dysregulated endocannabinoid signaling that may derive from pre-existing genetic factors or from prolonged drug exposure. Impaired endocannabinoid signaling contributes to dysregulated synaptic plasticity, increased stress responsivity, negative emotional states, and craving that propel addiction. Understanding the contributions of endocannabinoid disruptions to behavioral and physiological traits provides insight into the endocannabinoid influence on addiction vulnerability.

The brain reward system is critical for survival. The hedonic effects produced by eating, exercise and sexual activity provide important motivational effects that increase the likelihood of future engagement in these critical activities (e.g. positive reinforcement). The reward system is also essential for important negative hedonic responses in which aversive or unpleasant events (e.g sickness, bodily harm) increase the likelihood of behaviors that will avoid or relieve these negative states (e.g. negative reinforcement).

Seminal discoveries demonstrating that the effects of cannabis are mediated by cannabinoid receptors in the brain propelled significant research initiatives that expanded knowledge about the body's endogenous cannabinoid system (termed the endocannabinoid system; ECS) that is now acknowledged to play a prominent role in modulating brain reward function and maintaining emotional homeostasis. This Review examines the evidence for an endocannabinoid (EC) influence in the positive reinforcing effects of natural rewards and drugs of abuse. In contrast to the initial pleasurable experience of rewarding stimuli, prolonged drug exposure contributes to aberrant synaptic plasticity, negative emotional states and impaired learning and memory processes that sustain compulsive drug consumption characteristic of the addicted state. We explore dysregulated ECS signaling underlying these maladaptive processes and provide an overview of the existing literature

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regarding genetic factors associated with the ECS to gain insight about the potential contribution to addiction disorders.

## Endocannabinoid system and reward circuits

The ECS is comprised of G protein-coupled receptors, small neuromodulatory lipid ligands and biosynthetic and metabolic enzymes for the synthesis and degradation of the ligands, respectively. Two major types of cannabinoid receptor have been characterized and cloned: $CB_1$ and $CB_2$. $CB_1$ receptors ($CB_1$Rs) are the most abundant G protein-coupled receptor expressed in the adult brain, with particularly dense expression in regions with known involvement in reward, addiction and cognitive function including amygdala, cingulate cortex, prefrontal cortex (PFC), ventral pallidum, caudate putamen, nucleus accumbens (NAc), ventral tegmental area (VTA), lateral hypothalamus[1, 2]. $CB_2$ receptors ($CB_2$Rs) are mainly expressed by immune cells with recent evidence also suggesting $CB_2$R expression in neurons, glia and endothelial cells in brain[3]. $CB_1$R and $CB_2$R are coupled to similar transduction systems primarily through $G_i$ or $G_o$ proteins. $CB_1$Rs directly inhibit the release of GABA, glutamate and acetylcholine that produce widespread effects on neural signaling across many neurotransmitter systems.

At present the best characterized endocannabinoid (EC) ligands are N-arachidonylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG). Due to their lipid nature AEA and 2-AG are not stored in vesicles but are synthesized on an “on demand” basis by cleavage from membrane precursors and immediate release through $Ca^{2+}$-dependent mechanisms. AEA is derived from the phospholipid precursor N-arachidonoyl-phosphatidylethanolamine (NAPE), and while the precise mechanisms for AEA formation are not known, a very likely candidate is through N-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD). 2-AG derives primarily from the hydrolytic metabolism of 1,2-diacylglycerol (DAG) by sn-1-selective DAG lipases (DAGLα, DAGLβ). AEA is primarily catabolized through fatty acid amide hydrolase 1 (FAAH1) and 2-AG is catabolized through monoacylglycerol lipase (MAGL) and to a lesser extent α,β-hydrolase 6 (ABHD6), cyclooxygenase 2 (COX2) and FAAH1. The EC catabolic enzymes have distinct cellular anatomical locations with MAGL localized predominantly in presynaptic terminals and FAAH1 to the postsynaptic domain of neurons (Figure 1). AEA and 2-AG each exert agonist activity at $CB_1$R and $CB_2$R. AEA binds with slightly higher affinity to $CB_1$R vs. $CB_2$R, and like Δ9-tetrahydrocannabinol (Δ9-THC, the main psychoactive component of the cannabis plant) AEA exhibits low efficacy as an agonist at both receptors, producing sub-maximal signaling upon binding. 2-AG binds with essentially equal affinity at $CB_1$R and $CB_2$R and exhibits greater agonist efficacy than AEA. AEA and 2-AG also exhibit agonist properties at several secondary receptors including peroxisome proliferator-activated-receptors (PPARs), GPR55 and GPR119, and AEA exerts potent agonist effects at transient receptor potential ion channels including TRPV1.

## Neurobiology of reward

Mesocorticolimbic dopamine (DA) pathways, which arise from the midbrain ventral tegmental area (VTA), play a critical role in the mediation of reward. In particular, the VTA

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DA projection to the nucleus accumbens (NAc; also known as the ventral striatum) plays a prominent role in positive reinforcement (Figure 2), i.e. the recognition of rewards in the environment and promotion of goal-directed behavior (that is, 'approach behaviour') resulting in reward acquisition. Natural rewards such as food, sex and exercise and drugs of abuse including psychostimulants (such as cocaine and amphetamine), nicotine, alcohol, opiates and cannabinoids each increase NAc DA and this neurochemical response contributes to subjective reward and positive reinforcement[4]. Other components of the **limbic system** are also innervated by VTA DA neurons including the amygdala, hippocampus, orbitofrontal cortex and aspects of the prefrontal cortex (PFC). These regions are interconnected in complex circuits that involve excitatory (primarily glutamatergic) and inhibitory (primarily GABAegic) projections[5]. In broad, simplistic terms amygdala circuits contribute to the formation of associative reward- and fear-related memories, hippocampal circuits are critical for **declarative memory** functions and frontal cortical circuits mediate control of executive functions. In turn, innervation of the NAc by each of these circuits allows sensory and emotional information to be converted into motivational actions through the output to extrapyramidal motor systems. DA signaling in the dorsal striatum does not have a major influence in processing acute reward but plays a key role in the development of compulsive forms of reward seeking and consumption[6].

These same circuits participate in negative reinforcement mechanisms that promote behaviors for avoiding or relieving aversive states. In general, NAc DA levels are decreased by aversive conditions such as unavoidable shock, chronic pain, certain patterns of over- or under-eating and withdrawal from addictive drugs and the resultant increased activity of medium spiny output neurons contributes to aversive states[5, 7]. Negative reinforcement mechanisms associated with abstinence from long-term access to palatable food or abused drugs are mediated in part by excessive influence of pro-stress signaling systems (corticotropin releasing factor, dynorphin) and impaired function of anti-stress signaling systems (Neuropeptide Y, nociceptin) in stress circuits involving the central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST), frontal cortex and medial shell of the NAc[5, 8].

Thus, reward processing is mediated in large part through an interconnected network of structures including the VTA, NAc, ventral pallidum, CeA, BNST and PFC. In addition to the well-known involvement of DA described above, reward processing is also heavily influenced by many other systems including the cholinergic, opioid peptide, glutamatergic, and GABAergic systems. $CB_1$Rs are present in each of the interconnected structures involved in reward[1, 2, 9] where they exert widespread modulatory influences on excitatory and inhibitory signaling in a manner that influences reward processing[10, 11] In particular, ECs play a prominent role in fine-tuning the activity of the VTA-NAc DA projection and its influence on approach and avoidance behaviors that govern reward acquisition (Figure 3).

## Endocannabinoid signaling and reward

Both exogenous AEA and 2-AG increase extracellular DA levels in the NAc in a $CB_1$R-dependent manner[12] and the ECS exerts a strong influence on the fine-tuning of midbrain DA cell activity[13]. Through these and other interactions the ECS has a prominent influence

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on the hedonic effects of natural rewards such as food[14], sexual activity[15], and social interaction[16]. This is mediated in part through a direct $CB_1R$ modulation of the mesolimbic DA response to natural reward[16] and through the interactions between the ECS and other signaling systems (endogenous opioids, hypothalamic signaling molecules, etc.)[17, 18].

The rewarding effects of cannabinoid receptor activation are underscored by the fact that cannabis is one of the most widely used illicit substances worldwide. Δ9-THC is the primary psychoactive constituent in cannabis, and exhibits low efficacy as an agonist at $CB_1R$ and $CB_2R$[19]. In animal models both Δ9-THC and synthetic $CB_1R$ agonists enhance brain reward function (indexed by **intracranial self-stimulation**), produce rewarding effects in the **conditioned place-preference (CPP) paradigm** and are voluntarily **self-administered** (intravenously and also directly into the NAc shell and posterior VTA)[20]. These effects are critically reliant on $CB_1R$ signaling and are highly dose-sensitive with a rapid shift to negative reinforcing effects with increasing dose.

In contrast to the effects of exogenous CB agonists, pharmacological enhancement of EC levels generally does not produce rewarding effects *per se*. For example, in most animal studies, selective EC clearance inhibitors do not support operant self-administration, do not produce conditioned place-preference and do alter brain stimulation reward thresholds in rats and mice[21]. Similarly, neither exogenously administered AEA nor 2-AG, nor selective FAAH or MAGL inhibitors produce Δ9-THC-like **discriminative stimulus** effects. However, exogenous AEA and 2-AG each support operant self-administration in squirrel monkeys[21] and produce rewarding and Δ9-THC-like effects in rats when administered following EC clearance inhibition[9, 22]. Concurrent FAAH and MAGL inhibition in mice produces Δ9-THC-like discriminative stimulus and behavioral effects[22, 23]. These findings suggest that robust engagement of EC signaling is needed to evoke rewarding effects. However, recent evidence indicates that squirrel monkeys with a history of AEA, nicotine or cocaine self-administration will self-administer the FAAH inhibitor URB694, though this compound does not produce Δ9-THC- or nicotine-like discriminative stimulus effects and does not increase mesolimbic dopamine release[24]. Although it remains to be determined whether URB694 will be self-administered by drug-naïve monkeys or other species, this observation indicates that FAAH inhibition is not aversive and may produce mildly rewarding effects.

## CB receptor involvement in non-cannabinoid drug reward

The presence of $CB_1Rs$ throughout brain reward circuits and the rewarding effects produced by $CB_1R$ activation allows for the possible influence of EC signaling on the acute rewarding effects produced by non-cannabinoid substances. The effects of $CB_1R$ and FAAH manipulations on non-cannabinoid drug reward are summarized in Table 1. In general, drugs that activate $CB_1Rs$ do indeed appear to facilitate the rewarding effects of non-cannabinoid drugs. $CB_1R$ agonists increase the motivational and reinforcing effects of alcohol, nicotine and opiates indexed by animal models of drug reward including the CPP and operant self-administration assays while diminished $CB_1R$ signaling (either genetic deletion or pharmacological antagonism) attenuates the motivational and rewarding effects of these drugs[11, 25, 26]. The effects of $CB_1R$ antagonism on alcohol and nicotine reward result in part

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from a diminished ability of these drugs to increase NAc DA release[27]. Blockade of $CB_1$Rs specifically in the VTA decreases both alcohol and nicotine self-administration[28, 29] and blockade of $CB_1$Rs specifically in the NAc reduces alcohol consumption[28, 30]. However, while nicotine reward is critically dependent on the mesolimbic DA system[31], the motivational and rewarding effects of alcohol and opiates are less DA-dependent[32, 33] and the $CB_1$R modulation of the rewarding effects of these drugs likely involves non-dopaminergic mechanisms. Indeed, $CB_1$R antagonism does not alter opiate-induced increases in NAc DA but reduces opiate reward through prevention of opiate-induced reductions in ventral pallidal GABA release[34]. In comparison to these drugs, the effects of $CB_1$R manipulations on psychostimulant reward are modest and less consistent. $CB_1$R agonists reduce the facilitation of brain stimulation reward produced by cocaine and reduce cocaine self-administration[35, 36]. Most reports indicate that $CB_1$R antagonism does not affect psychostimulant reward (cocaine-induced enhancement of brain stimulation reward, CPP, self-administration) or cocaine-induced increases in NAc DA[37] (but see; [27, 37]).

Recent evidence in mice also implicates $CB_2$Rs in the modulation of drug reward, including an inhibitory influence on cocaine and alcohol reward[38, 39] but a facilitatory influence on nicotine reward[40, 41]. However, disparate observations have been made in rats[39, 42, 43] and it is possible these findings are influenced by species differences in $CB_2$R gene splicing that confer district $CB_2$R structure, function or pharmacology[39].

## Alterations in brain EC levels by drugs of abuse

Given the “on demand” nature of EC production and associated modest EC signaling tone under baseline conditions, the robust influence of CB receptor signaling on non-cannabinoid drug reward has led to the hypothesis that drug exposure increases brain EC formation. Substantial evidence demonstrates alcohol-induced alterations in postmortem EC content in rodent brain, though inconsistencies among studies cloud definitive conclusions regarding the direction of change and regional nature of the effects [44]. For example, alcohol exposure increases extracellular 2-AG levels in rat NAc (measured by *in vivo* microdialysis), and this is more pronounced following voluntary self-administration as compared to **non-contingent** alcohol exposure[28, 30]. In contrast, extracellular AEA levels in the NAc are unaltered by alcohol self-administration and decreased by non-contingent alcohol administration. Alcohol also appears to induce region-specific changes in brain tissue EC levels, with alcohol-induced disruptions consistently observed in striatal regions[30, 45, 46] but not frontal cortical areas[28] consistent with evidence that alcohol consumption is reduced by $CB_1$R antagonism in the VTA and NAc, but not PFC[28, 30, 47].

Similar to alcohol, nicotine also alters EC levels in rodent brain with factors such as the brain region evaluated and the voluntary nature of drug exposure having important relevance to the effects observed. Repeated non-contingent nicotine injections increase AEA levels in rat limbic forebrain and dorsal striatal tissue, but decrease both AEA and 2-AG levels in cortical tissue[48]. Intravenous nicotine self-administration increases extracellular levels of both AEA and 2-AG in rat VTA, and the effect on 2-AG is sensitized by chronic nicotine exposure[49]. Interestingly, while VTA 2-AG levels are elevated by either voluntary or non-contingent nicotine exposure, VTA AEA levels are increased only by voluntary nicotine

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self-administration[49]. Together with the evidence of distinct patterns of brain EC levels induced by volitional vs. non-contingent alcohol exposure[28, 30] these data suggest that brain EC production is influenced not only by drug-related pharmacological effects but also by neural activity engaged by active drug self-administration (possibly related to the motivation for drug consumption).

Relatively less is known regarding the effects of other rewarding drugs on brain EC levels. Available evidence consistently indicates that opiates increase AEA, but decrease 2-AG tissue concentrations in the striatum, limbic forebrain and hippocampus.[50, 51] Similarly heroin self-administration increases extracellular AEA with a concomitant decrease of extracellular 2-AG levels in the rat NAc[30]. Psychostimulants generally produce modest disruptions in brain EC content with subtle increases and decreases in 2-AG concentration in forebrain following non-contingent acute and chronic cocaine exposure, respectively (no other alterations evident regardless of region analyzed)[48, 52]. Moreover, voluntary cocaine self-administration does not alter rat extracellular NAc EC levels[30] but decreases 2-AG content in frontal cortex and hippocampal tissue[53–55].

Collectively these findings indicate that alcohol, nicotine, and opiates alter brain EC content, consonant with the $CB_1R$ influence on the behavioral effects produced by these drugs. The generally modest effects of psychostimulants on brain EC levels are in line with the subtle $CB_1R$ influence on psychostimulant-induced behaviors. Similar to that seen with multiple biological conditions, drug exposure often produces distinct and sometimes opposite effects on brain AEA and 2-AG levels, respectively. This suggests differential regulation of the synthesis and/or degradation of these EC moieties at specific synapses that may arise from the segregation of MAGL and FAAH in the pre- and post-synaptic compartments[56], or the hypothesized role of AEA and 2-AG in regulating ‘tonic’ and ‘phasic’ signaling in the EC system, respectively[57]. Although a general picture of drug-induced alterations in brain EC levels is emerging, experimental differences between studies including drug dose, method of drug exposure and the duration of treatment make it difficult to draw strong conclusions and additional studies are warranted.

## Influence of EC tone on drug reward

ECs are rapidly degraded, and thus strategies that reduce EC clearance have been employed as a means to further investigate the EC influence on drug reward. Most investigations have focused on the effects of FAAH inhibition because selective tools for inhibiting MAGL and other EC clearance enzymes were not available until recently. Such studies have shed light on important species differences that confounds the overall conclusions that can be made from existing data. For example, FAAH inhibition in mice increases nicotine reward in the CPP paradigm[58, 59], though in rats FAAH inhibition prevents nicotine-induced CPP, diminishes nicotine self-administration and blunts nicotine-induced increases in NAc DA release[60]. The potentiation of nicotine reward in mice by FAAH inhibition is $CB_1R$-mediated, whereas the reduction in nicotine reward in rats results from activation of PPAR-α by non-cannabinoid lipids such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) that are hydrolytically cleared by FAAH[61]. FAAH inhibition also produces distinct species-related alterations in alcohol consumption, with increased intake observed in mice

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but not rats[62–65]. The mechanisms underlying these differences are not understood. Brain region-specific disruptions in FAAH activity may be an important factor. In regard to alcohol reward as inhibition of FAAH activity specifically in the PFC results in increased alcohol consumption, and rats selectively bred for high alcohol intake and preference are characterized by reduced FAAH activity specifically in the PFC[64, 65]. The effects of FAAH inhibition on opiate and psychostimulant reward have primarily been studied in rats. FAAH inhibition does not alter morphine- or cocaine-induced disruptions in VTA DA cell firing or the self-administration of either drug[66, 67]. However, FAAH inhibition diminishes cocaine-induced alterations in NAc medium-spiny neuron activity[103] and this may contribute to enhanced sensitization of both cocaine-induced motor activity and mesolimbic dopamine responses following repeated cocaine exposure[68]. Other studies have investigated the effects of putative EC transport inhibitors such as AM404 and VDM11, and the findings thus far suggest that these compounds produce subtle and inconsistent effects on nicotine and cocaine reward[69–72].

While growing evidence implicates ECS influences in the modulation of acute drug reward, additional efforts are needed to further clarify the nature of EC disruptions caused by different classes of abused drug and the neural mechanisms through which these EC influences are mediated. Selective inhibitors of 2-AG clearance have recently been developed, but studies are still in their infancy so there are presently no published reports on the effects of enhanced 2-AG tone on drug reward and related physiological events. As such there remains a substantial gap of knowledge given the prominent 2-AG influence on neural signaling and plasticity related to both drug and natural rewards. Nevertheless, the role of the $CB_1R$ in drug reward is unequivocal and although there is evident complexity related to the effects produced by EC clearance inhibition (producing discrete modulation of EC tone in specific synapses/circuits as compared with broad $CB_1R$ activation by exogenous $CB_1R$ agonists) the extant evidence strongly supports an EC influence on the sensitivity to and motivation for several drugs of abuse.

## Endocannabinoid Signaling in Addiction

A number of factors influence the transition from intermittent, controlled drug use to the compulsive forms of drug-seeking and drug-taking that characterize addiction. Substantial evidence implicates genetic influences in the development of substance use disorders (SUDs) and pathological forms of eating, sexual behavior and gambling[73] and it is increasingly recognized that **epigenetic mechanisms** drive lasting changes in addiction-related gene expression[74]. Long-term drug exposure induces lasting neuroadaptations in motivational mechanisms that propel drug-seeking and use. Although initial drug use is motivated by hedonic processes, prolonged drug exposure progressively blunts reward system function thereby leading to escalated frequency and amount of drug consumption resulting in a dependent state wherein negative affective symptoms emerge during abstinence (e.g. dysphoria, anxiety, irritability). These negative emotional states arise from the recruitment of stress signaling systems (such as corticotropin releasing factor and dynorphin) and dysregulation of mechanisms that constrain these responses (such as neuropeptide Y and nociceptin) within the **extended amygdala**[5]. Renewed drug consumption alleviates these negative affective states, and this is conceptualized to motivate

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compulsive drug use through negative reinforcement[5]. Superimposed on these processes is a dysregulation of corticostriatal mechanisms mediating stimulus-response learning, constraint of impulsivity, **conditioned reinforcement** and incentive motivation resulting in a narrowed focus on drug-seeking at the expense of natural rewards[6]. ECs exert prominent modulatory influence in the extended amygdala and corticostriatal circuits[5, 32], and increasing evidence suggests that pre-existing genetic influences on the ECS and/or drug-induced dysregulation of EC function participates in the development and maintenance of addiction, including pathological forms of eating (Box 1). The following sections consider the consequences of chronic drug exposure on EC signaling within the reward circuitry and related disruptions in synaptic plasticity, affective state and learning and memory mechanisms related to extinction and relapse. Lastly, evidence for an influence of innate disruptions in ECS function (EC gene polymorphisms) as vulnerability factors for substance abuse and addictive disorders in humans is discussed.

**Box 1**

### Endocannabinoid signaling and eating disorders

Food and drug addiction derive in part from aberrant brain reward function and share overlapping neuroadaptations in the mesolimbic system[176]. Similar to drug addiction, food addiction is defined by compulsive over- or under-eating, aberrant feeding despite negative consequences and unsuccessful attempts to “normalize” dysfunctional eating.

The rewarding effects of self-starvation or binge eating may involve disrupted EC function[177]. Anorexia nervosa and binge eating disorder are associated with increased blood AEA levels and diminished levels of leptin[178] (an anorectic hormone that inhibits AEA synthesis). Anorexia is associated with a *CNR1* AAT repeat polymorphism[179] (but see[180]) and a synergistic association between the C385A FAAH gene and *CNR1* rs1049353 polymorphisms[181]. The C385A polymorphism is also associated with obesity[182], though it is unknown whether this may influence hedonic mechanisms, metabolism or both. Anorexia and bulimia nervosa are also associated with elevated plasma $CB_1R$ mRNA levels[183], increased $CB_1R$ availability in frontal cortical areas[184] and a nonsynonymous *CNR2* polymorphism[185]. Preclinical models of anorexia suggest therapeutic effects Δ9-THC[186] (but see[187]). Although two small clinical trials did not show Δ9-THC efficacy[176] a larger trial demonstrated small but significant weight gain in women with severe anorexia nervosa following 4 weeks treatment with Dronabinol[188]. Conversely, in binge eating disorder the $CB_1R$ antagonist Rimonabant significantly reduces binge eating and promotes weight loss with only modest presentation of psychiatric side effects[189, 190].

A withdrawal state similar to that associated with drugs of abuse is evident in rats during forced abstinence from highly palatable food[191, 192] including increased anxiety and excessive consumption upon renewed palatable food access. These symptoms result from increased $CRF_1$ signaling in the CeA[8] and are reversed by increased CeA 2-AG-$CB_1R$ signaling[193]. This suggests that dysregulated CeA function is a common factor

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contributing to pathological motivation for drugs and food, and that CeA EC signaling may counter withdrawal-related stress signaling.

## Chronic drug exposure and EC function

It is unsurprising that chronic cannabis use disrupts brain cannabinoid receptor availability and function. Using the *in vivo* technique of positron emission tomography (PET) imaging, Hirvonen *et al.*[75] reported down-regulation of brain $CB_1$Rs correlating with years of use by daily cannabis users, which were reversed after one month's monitored abstinence. Another PET study reported a global reduction in $CB_1$R availability[76] driven by differences in the temporal lobe, anterior and posterior cingulate cortex and nucleus accumbens. Similarly, animals given non-contingent chronic cannabinoid exposure exhibit decreased $CB_1$R function throughout the brain[77, 78]. Recent experiments employing **stochastic optical reconstruction microscopy (STORM)** demonstrate that chronic exposure to clinically-relevant doses of Δ9-THC results in a startling loss of $CB_1$Rs on terminals of perisomatically projecting GABA interneurons in the mouse hippocampus, and internalization of the remaining $CB_1$Rs[79]. The resulting deficits in inhibitory $CB_1$R control over hippocampal GABA release persisted during several weeks of Δ9-THC abstinence, and this may underlie the enduring loss of hippocampal LTP in rodents and memory deficits in humans evident following chronic cannabinoid exposure[80]. Surprisingly little is known of the effect of chronic cannabinoid exposure on other facets of ECS function. Chronic cannabinoid exposure increases enzymatic clearance of AEA and reduces brain tissue AEA content in rodents[81, 82] and frequent cannabis smokers present decreased AEA and increased 2-AG levels in blood[83, 84], though increased serum AEA levels are evident following at least 6 months of cannabis abstinence[85]. The contribution of these disruptions to cannabis use disorder and related physiological and behavioral disruptions is presently unexplored. However, as discussed below, ECs provide important homeostatic constraint over emotional state[86] and sleep function[87], and it's conceivable that Δ9-THC-induced impairment of endocannabinoid signaling contributes to negative emotional states and sleep disturbances present during protracted cannabis abstinence[88, 89].

Several findings support the hypothesis that chronic exposure to non-cannabinoid drugs disrupts EC signaling and processing. Chronic alcohol exposure in rodents alters EC-related gene expression in a manner sensitive to the intermittent nature of alcohol exposure and post-alcohol abstinence period[90] and down-regulates $CB_1$R expression and function[45, 91]. Post-mortem studies of alcohol dependent humans also demonstrate disrupted $CB_1$R expression in the ventral striatum and cortical regions[92] and *in vivo* imaging studies demonstrate decreased $CB_1$R availability in heavy drinking alcoholics that persist for at least 1 month of abstinence[93, 94] (but see[95]). Although a potential contribution of *CNR1* gene variants to these observations cannot be excluded, a common interpretation based on animal studies is that these $CB_1$R adaptations in alcoholic humans are a consequence of prolonged alcohol-induced increases in brain EC levels. This is supported by evidence of transient recovery (and perhaps eventual upregulation) of $CB_1$R function in humans during protracted alcohol abstinence[91, 96]. In rodents, chronic nicotine exposure induces distinct age-related disruptions in $CB_1$R binding, with increased levels evident in the PFC, VTA and

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hippocampus of adolescent but not adult rats[97], and increased hippocampal and decreased striatal $CB_1R$ binding in adult rats during protracted nicotine abstinence[98]. Few studies have investigated altered $CB_1R$ binding following chronic opiate or psychostimulant exposure but findings in rodents implicate impaired $CB_1R$ function in the development and expression of opiate dependence[99, 100] and demonstrate that chronic cocaine increases $CB_1R$ binding in dorsal striatum, NAc and cortical areas[101]. Interestingly, detoxified cocaine addicts present significant increases in plasma AEA and decreases in plasma 2-AG content[102] but the functional consequence of these disturbances is not known. Overall, accruing data suggests that long-term exposure to a variety of drug classes compromises EC processing and $CB_1R$ expression and function. As discussed below these perturbations may contribute to aberrant neural signaling during acute and protracted drug abstinence.

## Addiction-related synaptic plasticity

The development and persistence of addiction is attributed to maladaptive **synaptic plasticity** evident in the neuronal reorganization (molecular, cellular and functional activity) of mesocorticolimbic and striatal pathways. EC signaling at $CB_1Rs$ is implicated in several forms of synaptic plasticity, most commonly (i) depolarization-induced suppression of excitatory or inhibitory transmission (DSE/DSI), (ii) short-term depression (STD) and (iii) long-term depression (LTD, a prolonged form of weakened synaptic strength)[103, 104]. STD and DSE/DSI are mediated primarily by 2-AG signaling, typically persist for a minute or less, and have been observed in brain areas relevant to reward and addiction including the VTA, basolateral amygdala, hippocampus, neocortex and substantia nigra[10]. In comparison, EC-mediated LTD can persist for hours to weeks, is particularly important in learning and memory, and has also been observed in addiction-related regions including the NAc, VTA, amygdala, PFC, hippocampus and dorsal striatum[10].

Acute and chronic alcohol exposure reduces $CB_1R$-dependent plasticity resulting in long-lasting disinhibition of striatal output neurons and diminished EC-mediated LTD at inhibitory striatal synapses[105, 106]. Because the dorsal striatum mediates reward-guided learning and habitual behavior these EC disruptions may contribute to maladaptive habitual behavior that perpetuates addiction[107]. Cocaine diminishes EC-LTD of excitatory transmission in the NAc[108] and facilitates EC-LTD of inhibitory signaling at VTA DA synapses[109, 110], resulting in diminished inhibitory control over VTA DA cell activity and heightened excitatory signaling in the NAc (Figure 4). Cocaine also disrupts EC-LTD of excitatory transmission in the BNST[111], a component of the extended amygdala, and this may contribute to aberrant stress-reward interactions (via projections to the VTA)[112] and excessive anxiety-like behavior. Similarly, chronic Δ9-THC or synthetic $CB_1R$ agonist exposure abolishes EC-LTD of excitatory and inhibitory signaling in the NAc and hippocampus[113, 114] which may significantly impact reward processing mediated by these regions. Little is known regarding opiate- or nicotine-induced disruptions in EC-mediated synaptic plasticity, though cue-induced reinstatement of nicotine-seeking behavior (an animal model of relapse) relies in part on the induction of $CB_1R$-mediated LTP of cortical synapses in the BNST[115]. Thus, chronic drug exposure disrupts EC-mediated forms of synaptic plasticity in several regions involved in reward processing. As will be discussed,

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impaired EC-mediated plasticity may also contribute to dependence-related affective disruptions that serve to sustain drug dependence.

### Withdrawal-related affective disruption

Stress plays a prominent role in the development of addiction[116] and stress exposure disrupts EC-mediated plasticity in regions including the NAc, amygdala and BNST[117] that participate in emotional control. Withdrawal from most drugs of abuse is associated with increased stress responsivity and persistent negative affective symptoms such as anxiety and depression, the severity of which are closely associated with relapse susceptibility. Comorbidity of affective disorders and SUDs is prevalent and preexisting negative affective traits may be an antecedent to addiction. The ECS participates in a negative feedback system that constrains emotional distress under stressful circumstances and contributes to the suppression of aversive memories[117, 118]. This function is reliant on EC-mediated forms of synaptic plasticity, and deficient EC signaling is associated with increased anxiety and depression. As such impaired EC function may contribute to negative affective states and increased stress responsivity that underlie negative reinforcement mechanisms driving drug use by dependent individuals and that contribute to drug relapse following periods of abstinence.

Mice lacking $CB_1$Rs exhibit greater anxiety-like behavior than normal animals during nicotine withdrawal[119], though innate anxiety-like behavior in the knockouts clouds interpretations. Studies evaluating EC clearance inhibition provide more direct insight into withdrawal-related EC disruption and negative affect. Acute FAAH inhibition reverses enhanced anxiety-like behavior normally present during both nicotine and alcohol withdrawal[65, 120] and the EC transport inhibitor AM404 attenuates depression-like behavior during nicotine withdrawal[121]. Post-traumatic stress disorder is particularly prevalent among individuals with alcohol-use disorders and this is often modeled in rodents using the fear-potentiated startle paradigm to study reflexive physiological reaction to a stimulus. Rodents selectively bred for high alcohol consumption exhibit greater fear-potentiated startle than corollary lines bred for low alcohol consumption[122, 123] and acute FAAH inhibition by LY2183240 reduces fear-potentiated startle in high alcohol-preferring, but not low alcohol-preferring mice[124] consistent with the efficacy of FAAH inhibition for accelerating the extinction of aversive memory[125]. LY2183240 also enhances the conditioned rewarding effects of alcohol without altering alcohol consumption itself, suggesting that FAAH inhibition influences memory-related processes (conditioned fear and conditioned alcohol reward) in animals predisposed toward high alcohol consumption.

**Addiction-related learning and memory—**Both positive and negative memories and conditioned cues associated with drug use perpetuate drug seeking and the continued cycle of abuse. The ECS plays a prominent role in learning and memory processes[126] and $CB_1$R signaling is strongly linked to the conditioned rewarding effects of alcohol, nicotine and opiates[11, 25]. Although drug-induced conditioning effects are generally interpreted in the context of drug reward, a $CB_1$R influence on the associative learning aspects of drug exposure is also possible, which as discussed below may have relevance to the persistent reactivity to drug-related memories that characterizes addiction.

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### Drug-seeking (relapse)

Drug exposure produces powerful interoceptive effects that become associated with environmental cues such that these cues alone can induce craving and promote relapse following periods of abstinence[127]. In addition to conditioned drug memories, acute exposure to a preferred drug or pharmacologically-related agent (e.g. drug priming) and stressful events can also precipitate relapse[116].

Animal models of relapse demonstrate an important cannabinoid influence on the reinstatement of extinguished drug-seeking and drug-taking behaviors. Δ9-THC and synthetic $CB_1R$ agonists reinstate drug-seeking for cannabinoids, alcohol, nicotine, opiates and cocaine while $CB_1R$ antagonists attenuate drug-seeking behavior associated with each of these drugs[25, 128, 129]. $CB_1Rs$ in the PFC and NAc shell influence cue-induced reinstatement of both heroin- and nicotine-seeking behavior, while $CB_1Rs$ in the basolateral amygdala also contribute to cue-induced nicotine-, but not heroin-seeking behavior[130, 131]. Despite the subtle effects of $CB_1R$ inactivation on psychostimulant self-administration, $CB_1R$ antagonism attenuates drug-primed, cue-induced and some forms of stress-induced reinstatement of cocaine- and methamphetamine-seeking behavior in rats[25]. Thus, $CB_1R$ signaling modulates drug-seeking for a variety of pharmacologically distinct drugs. There is also evidence that $CB_1R$ antagonism also blocks both cue- and “priming”–induced reinstatement of seeking behavior for non-drug rewards such as sucrose and corn oil[132, 133] (but see[134]). Accordingly $CB_1R$ signaling appears to participate in the modulation of conditioned reward in general.

Both drug-primed and cue-induced nicotine- and cocaine-seeking behavior are reduced following acute FAAH inhibition that leads to elevated AEA levels [25, 60], which may be surprising since $CB_1R$ agonists enhance both nicotine- and cocaine-seeking behavior[25]. However, inhibition of EC clearance likely amplifies EC signaling preferentially in circuits/synapses activated by a given stimulus (in this case drug-seeking behavior), rather than inducing more widespread indiscriminate $CB_1R$ activation as produced by exogenous $CB_1R$ agonists. Moreover, FAAH hydrolyzes a large variety of fatty acid moieties and the effects of FAAH inhibition on drug-seeking may involve non-cannabinoid lipid signaling. In this regard, it’s notable that the EC transport inhibitor VDM11 attenuates both nicotine- and cue-induced nicotine-seeking behavior[70] and this compound may preferentially block AEA clearance with weaker effects on non-cannabinoid lipids[135, 136]. Similarly, the EC transport inhibitor AM404 dose-dependently attenuates nicotine- and cue-induced nicotine-seeking behavior without altering nicotine self-administration[71].

In contrast to nicotine- and cocaine-seeking, neither FAAH inhibition nor EC transport inhibition alter cue- or stress-induced reinstatement of alcohol-seeking behavior[65, 137]. However, studies in human alcoholics suggest a relationship between EC tone and craving that may relate to the degree of dependence and possibly inherent factors contributing to alcoholism vulnerability. In social drinkers, alcohol-related cues increase both craving and plasma AEA levels and the relative magnitude of cue-induced increases in AEA is significantly correlated with the degree of craving[138]. However, recently detoxified alcoholics present significantly lower baseline plasma AEA levels than non-dependent social drinkers and while alcohol-related cues elicit more intense craving in alcoholics these

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subjects do not present significant cue-induced increases in plasma AEA. This blunted AEA response may reflect aberrant EC processing in alcoholics, but further investigations are needed to confirm a direct link between this potential peripheral biomarker and brain EC signaling, as well as possible causal relationships between dysregulated EC processing and behavior.

## Extinction learning

The potent motivational effects of drug-related cues create substantial difficulties during periods of attempted drug abstinence, and are causal in the reinstatement of drug intake (e.g. relapse)[127]. One approach for reducing the motivational impact of drug-associated cues is through extinction training wherein a subject learns that these cues no longer have predictive value. However, extinction therapy is generally ineffective for reducing relapse in both humans[139] and rodents[140], and it is conceivable this is a consequence of diminished learning mechanisms required to override the original cue association memory. The ECS plays a prominent role in memory extinction, and deficient $CB_1R$ signaling results in impaired extinction of cued fear memory, contextual fear memory, fear-potentiated startle and spatial memory under mildly aversive conditions[141, 142]. Moreover, as previously noted FAAH inhibition facilitates the extinction of fearful memory in mice selectively bred for high levels of alcohol preference and consumption[124]. Because aversive memory may be involved in relapse to drug taking[143] deficient EC signaling following long-term drug exposure may contribute to the limited efficacy of extinction therapy for addiction.

**EC gene polymorphisms and addiction—**Approaches to explore the contribution of the ECS to addiction disorders in humans often involve heritability considerations since it is now acknowledged that genetics play an important role in drug addiction vulnerability accounting ~30–80% for risk depending on the drug class[144, 145]. Based on the growing evidence of the ECS in regulating reward, mood and cognition and due to its prominent expression within neuronal systems related to these functions, the ECS has been viewed as a central target for candidate gene studies of addictive disorders. Similar to the preclinical animal studies described above, most investigations have focused on the *CNR1* and *FAAH* genes that encode the $CB_1R$ and FAAH proteins, respectively[146, 147]. Consistent with most genetic investigations, factors including race, ethnicity, type of drug, polysubstance use and population sample size are important confounds. Nevertheless, although not all equivocal, what can be garnered from existing genetic studies (though limited) suggests that genomic heterogeneity of the EC-related genes may influence in part substance abuse vulnerability and relate to behavioral and pathophysiological traits that are highly associated with addictive disorders in humans (Figure 5).

## CNR1

The human *CNR1* gene is located on the human chromosome 6 (6q14-q15) with the coding region situated at the 5′ end of exon 4. Several different *CNR1* isoforms vary in expression across brain regions, though each of the main mRNA variants express the same exon 4 that encodes the $CB_1R$ protein[148]. Indeed, the *CNR1* gene exhibits substantial functional conservation with few common missense variants in the $CB_1R$ protein[148].

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One of the first *CNR1* variants explored in relation to drug abuse was the (AAT)n triplet repeat microsatellite polymorphism in the 3′ UTR located close to the exon 4 translational start site[148]. Unfortunately direct functional evidence is lacking to understand its relevance of EC processing, but the increased number of repeats is speculated to result in reduced $CB_1R$ expression[149]. Increased frequency of long $(AAT)_n$ repeats was initially observed in an intravenous drug-dependent non-Hispanic USA Caucasian population[150], and this was partially supported in subsequent evaluations of Afro-Caribbean subjects[151]. Some reports failed to replicate the original finding[148, 152], but meta-analysis of multiple variants of the *CNR1* gene in Caucasian populations specifically identified the AAT polymorphism (n>16 repeats) as the only significant association with illicit substance use disorders[153]. Interestingly, the (AAT)n polymorphism has been linked with reduced amplitude of the frontal lobe P300 event-related brain potential, a disruption that has been suggested as a neurobiological endophenotype of impaired cortical processing in drug abusers[146].

Additional SNPs of the *CNR1* gene have been investigated, the most frequent of which is a silent intragenic biallelic polymorphism (G1359A; rs1049353). This exon 4 synonymous mutation does not change the amino acid sequence of the mature protein, but the SNP is speculated to affect mRNA stability or protein translation that could alter $CB_1R$ function. Several investigations, though not all congruent, suggest an association of the *CNR1* G1359A polymorphism with substance abuse. For example, the A-allele is associated with severe alcoholism, specifically in relation to enhanced withdrawal delirium in Caucasian patients[154] and enhanced impulsivity in Native Americans with a high lifetime prevalence of substance dependence[155]. The G1359A variant has also been associated with heroin abuse in Caucasians, but with the A-allele conferring protection and the G/G genotype addiction risk[156]. Additional studies are clearly needed to determine whether the risk/protection profile might depend on the drug class.

Aside from the G1359A SNP, most of the other *CNR1* variants reported to be associated with addiction are not within the coding region; this is not surprising considering that it is now evident that most variants in the genome falls outside of protein-coding regions[157]. The rs2023239 variant representing a C/T polymorphism in the intronic region upstream of exon 3 has been shown to relate to $CB_1R$ levels measured both in postmortem brain tissue[158] and *in vivo* using PET imaging[93], with the C-allele associated with enhanced $CB_1R$ levels in the normal human brain. As discussed above, increased CB1R in animal models is predictive of addiction vulnerability and indeed the rs2023239 SNP has been linked to a general liability for substance abuse[148]. C allele individuals use greater amounts of marijuana, exhibit higher marijuana dependency and experience greater negative affect and craving for marijuana following withdrawal[159]. The rs2023239 minor allele also associates with increased activation in reward-associated brain areas [measured by blood oxygenation level dependent (BOLD) imaging] to marijuana-related cues [160]. rs2023239 C-allele carriers also have enhanced alcohol cue-elicited brain activation in the PFC, NAc and midbrain (consistent with the VTA and surrounding regions), greater subjective reward when consuming alcohol, a strong correlation between cue-elicited brain activation and alcohol consumption measures, as well as a strong association with alcohol use disorder and craving measures[161].

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A number of *CNR1* **haplotype blocks** have also been linked with addiction. When analyzed as a haplotype (T-A-G), three SNPs (rs806379-rs1535255-rs2023239) in the distal region of intron 2 of the *CNR1* gene were significantly associated with polysubstance abuse in adults from different ethnicities[148]. Moreover, Agrawal and colleagues[162] demonstrated an association between a *CNR1* haplotype (rs806380, rs806368, and rs754387) and cannabis dependence (the majority of those subjects also met criteria for alcohol dependence). The rs806380 SNP proximal to the T-A-G haplotype has also been linked with the development of cannabis dependence symptoms (protective effect of G-allele). Other haplotypes have been reported to associate with either low (rs6454674, rs806380, rs806377, rs1049353: G-G-C-C) or increased (T-A-C-C and G-A-C-C) risk for cannabis dependence[163]. However, inconsistent and nominal significance have been reported in replication studies of cannabis dependence in adolescent and young adult populations[164] and for other haplotypes in substance abuse populations[165]. Overall, although the majority of genetic investigations suggest an association between *CNR1* variants and aspects of SUD, the data are not definitive and no causative loci have been described to date. What appears most consistent in the human genetic studies is a relevance to cue and craving which would complement the preclinical animal studies that demonstrate their direct link to the ECS.

## FAAH

In comparison to *CNR1*, few genetic investigations have focused on other components of the ECS with *FAAH* being the second gene most often studied in relation to addiction based on AEA's important functional role. The human *FAAH* gene is located on chromosome 1p35–34 with 15 exons and functional protein domains encoded across multiple exons. A SNP that has been highly investigated is rs324420, located in exon 3 that results in a missense mutation of a C-to-A replacement at position 385, leading to a proline to threonine change at protein position 129[166]. This C385A SNP is functional, and believed to result in reduced FAAH expression and enzyme activity such that individuals with the A/A genotype have enhanced plasma concentrations of AEA and other N-acylethanolamine FAAH substrates[167]. Although some studies have not observed associations between the C385A polymorphism with substance use disorders, existing evidence implicates this genetic disruption in addiction-related behaviors in different races and ethnicities[147]. Specifically, the A/A genotype associates with reduced vulnerability for cannabis dependence in adult Caucasians while the C/C genotype associates with increased craving and negative affect during marijuana withdrawal. Initial studies failed to detect a link between the A/A genotype with alcohol or nicotine dependence[168] though recently an over-representation of C/C carriers was observed among risky alcohol drinkers[169]. Carriers of the *FAAH* C385A SNP display increased ventral striatal reactivity associated with delay discounting, a behavioral index of impulsivity and reward sensitivity[170] and a markedly decreased relationship between threat-related amygdala reactivity and trait anxiety, similar to patterns observed in individuals with high familial risk for alcoholism[171]. These findings suggest that dysregulation of FAAH function through the C385A polymorphism confers increased impulsivity and increased anxiety sensitivity.

Collectively, recent studies of *CNR1* and *FAAH* genetic variants generally suggest an association with **endophenotypes** implicated in addiction susceptibility including reward

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sensitivity, impulsivity and negative affect consistent with preclinical evidence linking the ECS to such behaviors. Gene-gene interactions within the ECS may also be relevant to vulnerability as there appear to be additive interactions between variants of the *FAAH* (C385A/rs324420) and *CNR1* (rs2023239) genes resulting in heightened neural responses in reward-related brain areas to marijuana cues and more severe negative affect during marijuana abstinence[159, 160]. Growing evidence of an EC influence on epigenetic mechanisms suggests an additional but understudied way in which EC signaling may contribute to addiction (Box 2).Clearly, a major confound of most investigations to date is the small population size emphasizing the need for replication studies and larger populations. The few existing agnostic genome-wide approaches have not identified EC-related genes in relation to substance use disorders interrogated thus far. However, the contribution of endophenotypes along the continuum between genotype and drug abuse phenotype has not been interrogated despite the complex nature of non-Mendelian addictive disorders. The lack of systematic consideration of behavioral traits limits the possibility to understand the full repertoire of the ECS to individual vulnerability to addition. Moreover the functional consequences of the variants (causal or correlated) are unknown which makes definitive conclusions challenging.

**Box 2**

### Endocannabinoid influence on epigenetic mechanisms

Epigenetic influences refer to functionally relevant changes to the genome that do not involve disruptions in the nucleotide sequence of DNA. Examples of epigenetic mechanisms include DNA methylation, **posttranslational histone modification**, nucleosome positioning and small non-coding RNAs (microRNA, small interfering RNA (siRNA)). Recent evidence demonstrates that epigenetic factors can regulate the expression of EC-related genes, and that ECs may themselves induce epigenetic alterations[194]. For example, DNA hypermethylation of the *CNR1* gene results in down regulation of $CB_1R$ transcription in the CNS and immune system, while decreased DNA methylation can result in increased FAAH transcription; these processes have been implicated in several pathologies including colon cancer and late onset Alzheimer's Disease. Conversely, EC-induced alterations in enzymes influencing histone modification may disrupt transcription of several genes including those encoding various neurotransmitter systems. In rodents, early life stress (maternal separation) is associated with elevated DNA methylation of the *CNR1* promoter[195], which through a resultant decrease in $CB_1R$ expression could contribute to affective dysregulation and addiction susceptibility later in life. Several studies implicate increased epigenetic influences following chronic Δ9-THC exposure. Marijuana dependent patients present robust methylation of the *CNR1* promoter in association with diminished *CNR1* mRNA in peripheral blood cells[196]. Further, offspring of maternal marijuana users exhibit histone methylation and dysregulated mesolimbic dopamine $D_2$ receptor Drd2 expression[197] and adolescent Δ9-THC exposure is associated with NAc chromatin modifications and concurrent upregulation of the opioid neuropeptide proenkephalin gene[198]. Prenatal alcohol exposure increases expression of the regulatory microRNA miR-26b (which targets the 3′-UTR of the *CNR1* transcript) and decreased *CNR1* transcription in the adult

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mouse brain[199]. Thus, growing evidence suggests EC-related epigenetic influences following drug exposure.

## Summary and future directions

Although enhancement of EC levels does not produce rewarding effects *per se*, EC signaling at cannabinoid receptors participates in the mediation and modulation of both natural and drug-induced reward. Brain EC content is modulated by most drugs of abuse and natural rewards and a robust $CB_1R$ influence on the motivation to consume distinct classes of abused drugs and the association of *CNR1* gene polymorphisms with aberrant reward processing and addictive behaviors strongly implicates $CB_1Rs$ in the etiology of addiction. Long-term drug use leads to neuroadaptive down-regulation of EC signaling resulting from diminished $CB_1R$ and/or $CB_2R$ function as well as possible disruptions in EC biosynthesis and/or clearance. This blunting of EC function may contribute to increased stress responsivity, increased negative affect, inefficient extinction of drug-related memories and drug seeking/craving that are known contributors to relapse. Recent preclinical evidence demonstrates the efficacy of EC clearance inhibitors for ameliorating these behavioral abnormalities that might offer future therapeutic interventions for addiction disorders. Importantly, because ECs are generally produced in a synapse-specific manner, EC clearance inhibitors may preferentially facilitate EC signaling in specific circuits engaged by distinct stimuli (e.g. stress, drug-associated cue, etc.) and therefore could present fewer unwanted behavioral effects than produced by exogenous agonists that produce widespread CB receptor activation.

Despite growing attention on cannabinoids, there continues to be notable gaps in our understanding of the EC influence on reward and addiction. The ECS plays a prominent role in neuronal guidance and brain development[172] and as such disruptions in EC function at an early age likely has substantial consequences for adult brain function. This is underscored by increasing evidence of the long-term consequences of prenatal or adolescent cannabinoid exposure [173, 174]. Though the effects of early life exposure to non-cannabinoid drugs are well studied, the specific contributions of persistent drug-induced disruptions in EC signaling on adult neural function and behavior are not understood. Robust bidirectional interactions between the ECS and sex hormones is now recognized[175], but few studies have characterized possible sex differences in the EC influence on reward function, addiction and cognitive processing. There are also substantial limitations in the interpretation and replication of genetic analyses of the EC influence in addiction due to heterogeneity of the populations studied, drug class, polysubstance use and even drug use phenotypes examined. Large-scale future studies across different populations and drug classes will be critical to understand the relative impact and causal nature of ECS-related genetic mutations in the vulnerability to addictive disorders. Filling these gaps of knowledge are critical given the important need for scientific data to help guide current discussions and changes being made in marijuana legalization policies.

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## Glossary of Terms

(note entries with asterisks may be cut as needed)

| | |
|---|---|
| **Limbic system** | A collection of brain structures that includes the amygdala, hippocampus, limbic cortex, limbic midbrain areas and anterior thalamic nuclei, and regulates autonomic and endocrine function and participates in the control of emotion, motivation, long-term memory and olfaction |
| **Intracranial Self-Stimulation (ICSS)** | An operant behavioral paradigm in which subjects elicit a behavioral response (level press, wheel turn) to receive brief electrical pulses into specific regions in the brain reward pathways |
| **Conditioned Place-Preference (CPP) procedure** | Behavioural paradigm to study the rewarding and aversive effects of drugs associated with a specific environment |
| **Self-administered** | Operant self-administration is a behavioral procedure in which experimental subjects learn to elicit an operant response (lever press, nose poke) to receive a drug reward (intravenous infusion, small bolus for oral consumption, delivery of discrete bolus of vapor that is inhaled) |
| **Discriminative stimulus** | a stimulus in a drug discrimination paradigm that the animal has learned to associate with predictable consequence (rewarding or unrewarding) and that increases the elicitation of a specific behavior in |
| **Non-contingent** | Drug delivery that is involuntary (experimenter-administered) or not contingent on the elicitation of a behavioral response by an experimental subject; sometimes referred to as forced administration |
| **Epigenetic mechanisms** | the mechanisms by which functionally relevant changes to the genome occur that do not involve disruptions in the nucleotide sequence of DNA, and include DNA methylation, histone modification and non-coding RNA-associated gene silencing |
| **Extended amygdala** | A grouping of brain regions involved in processing reward perception that includes the central nucleus of the amygdala, sublenticular substantia innominate, nucleus accumbens shell and the bed nucleus of the stria terminalis |

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|---|---|
| **Conditioned reinforcement** | The process through which neutral stimuli acquire motivational properties through association with a primary reinforcer |
| **Stochastic optical reconstruction microscopy (STORM)** | A super-resolution imaging technique that uses sequential activation and time-resolved localization of photoswitchable fluorophores to create high resolution images enabling the precise flurophore localization with nanometer resolution |
| **Synaptic plasticity** | The process by which synaptic communication strengthens or weakens as a result of changes in morphology, composition or signal transduction efficiency in response to intrinsic or extrinsic signals |
| **Haplotype block** | A set of DNA variations, or polymorphisms, that tend to be inherited together |
| **Endophenotype** | A term used to separate behavioral symptoms into stable phenotypes with a clear genetic basis, typically applicable to heritable disorders |
| **Posttranslational histone modification** | A covalent modification of proteins that package and order DNA into nucleosomes (e.g. histones). These modifications occur during or after histone biosynthesis |
| **Cytogenetic Band** | Distinct region on the chromosome (visible microscopically after special staining) |

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## Biographies

**Prof Loren Parsons**

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Loren Parsons received his Ph.D. in chemistry from Emory University in Atlanta, Georgia, USA followed by postdoctoral training in behavioral pharmacology and neurochemistry with George Koob and Friedbert Weiss at The Scripps Research Institute (TSRI) in La Jolla, California, USA. He is currently Professor in the Committee on the Neurobiology of Addictive Disorders at TSRI, Director of the TSRI Alcohol Research Center and Director of the Ruth L. Kirchstein Training Program at TSRI. His research focuses on the neurobiological bases of addiction with particular focus on the role of dysregulated endocannabinoid signaling in drug dependence and protracted withdrawal, and the cortical mechanisms underlying dependence-related cognitive impairment. The Parsons lab website is http://www.scripps.edu/parsons/Research.html.

**Prof Yasmin Hurd**

Yasmin Hurd obtained her Doctor of Medical Science degree (PhD) at the Karolinska Institute, Stockholm, Sweden and conducted her post-doctoral training at the National Institute of Mental Health, Bethesda, Maryland, USA. She is currently Professor of Psychiatry, Neuroscience and Pharmacology & Experimental Therapeutics at the Icahn School of medicine at Mount Sinai in New York where she is also the Ward-Coleman Chair of Translational Neuroscience and Director of the Center for Addictive Disorders. Her laboratory investigates the developmental and cross-generational effects of cannabinoids as well as epigenetic mechanisms and genetic factors contributing to addiction risk. The Hurd lab website is http://neuroscience.mssm.edu/hurd.

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## Online summary

- Cannabinoid receptors and their endogenous ligands are widely expressed throughout the brain, with particularly strong presence and influence in neuronal circuits such as the mesocorticolimbic pathways highly implicated in reward and addiction.
- $CB_1$ receptor signaling influences the motivation for both natural and drug rewards. In comparison to most drugs of abuse $CB_1$ receptors exert only modest influence on psychostimulant intake.
- Brain EC levels are increased by most drugs of abuse, though the nature of this effect differs between classes of drugs and across brain regions. The response contingency of drug exposure (volitional vs. response-independent) appears to influence brain EC production, suggesting contributions of both drug-related pharmacological effects and neural activity engaged by active drug-seeking.
- Chronic exposure to drugs of abuse generally results in impaired $CB_1$ receptor function, loss of EC-mediated synaptic plasticity in addiction-related neural circuits, and negative affective states that can be ameliorated through pharmacologically enhanced EC tone. The ECS plays a strong role in modulating relapse-like behavior induced by conditioned cues or reward priming, and this is evident for both natural and drug rewards.
- Recent investigations of *CNR1* and FAAH gene variants generally suggest an association with endophenotypes implicated in addiction susceptibility including reward sensitivity, impulsivity and negative affect. However, confounding factors including restricted sample size, ethnicity and polysubstance use limit interpretational power, and the functional consequences of the variants (causal or linked) are currently unknown.

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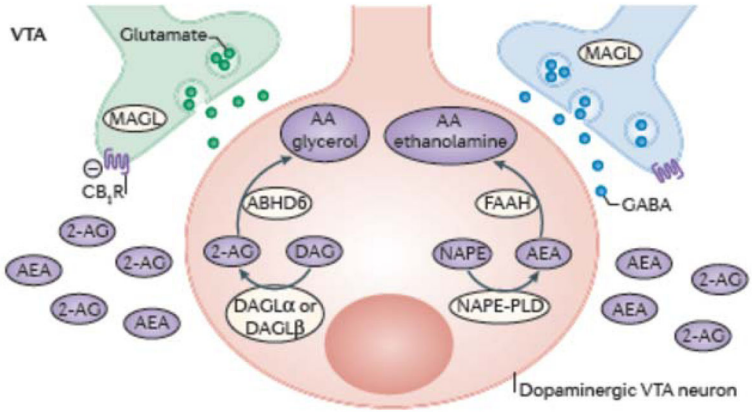


**Figure 1. Endocannabinoid biosynthesis, signaling and clearance**

The most commonly accepted route for AEA synthesis is from catalysis of *N*-arachidonoyl-phosphatidylethanolamine (NAPE) via a specific phospholipase D (NAPE-PLD). 2-AG derives from the hydrolysis of 1,2-diacylglycerol (DAG) via the sn-1-selective DAG lipases DAGLα and DAGLβ. DAGLα is found on the plasma membranes of both dopaminergic and non-dopaminergic neurons in the VTA, opposite $CB_1R$-expressing glutamate and GABA axon terminals[200]. Termination of EC signaling is initiated by cellular reuptake followed by enzyme-mediated hydrolytic cleavage. 2-AG hydrolysis is primarily mediated by presynaptic monoacylglycerol lipase (MAGL), though post-synaptic enzymes including ABHD6 also contribute to 2-AG clearance. AEA hydrolysis occurs in postsynaptic cells through fatty acid amide hydrolase (FAAH). Although these mechanisms are depicted here in the VTA, the pre- and post-synaptic organization of EC biosynthetic and hydrolytic enzymes is generally conserved throughout the brain.

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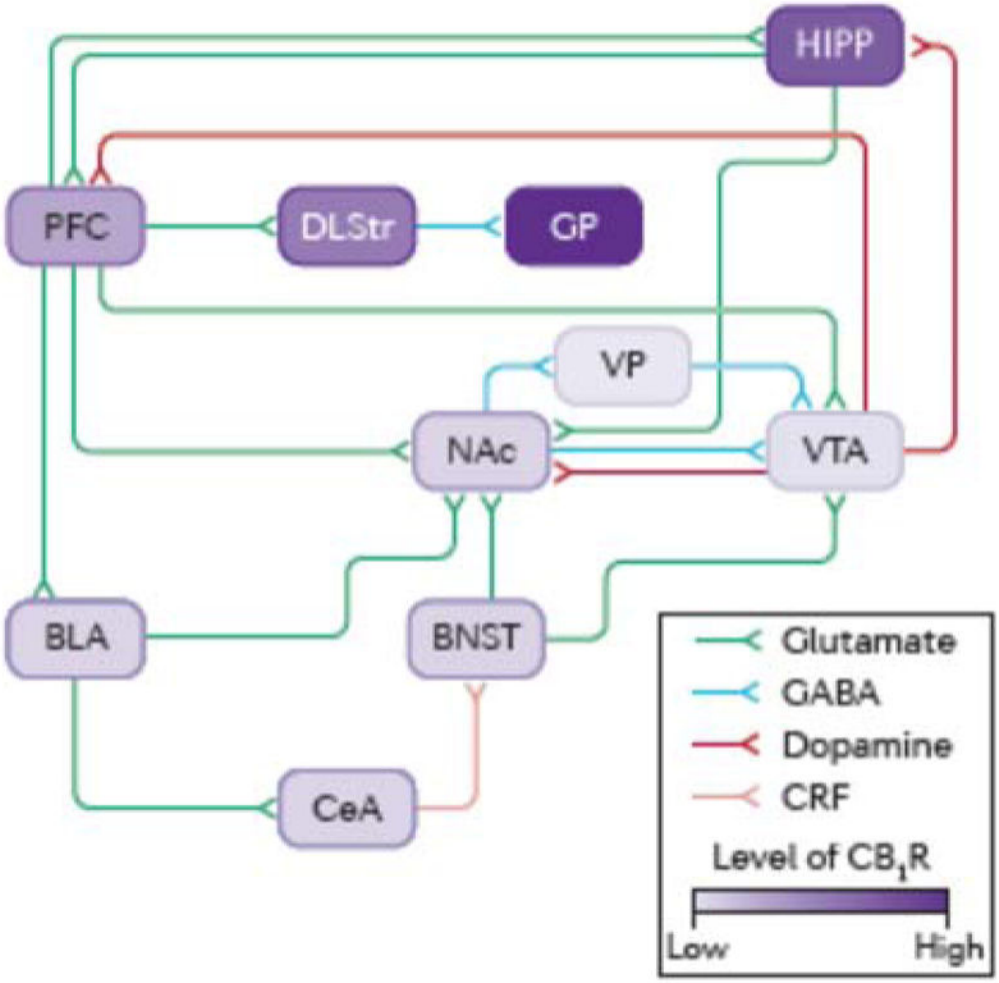


**Figure 2. Distribution of EC signaling mechanisms within the brain reward circuits**

$CB_1$Rs are expressed throughout the regions implicated in reward and addiction including the basolateral amygdala (BLA), prefrontal cortex (PFC), hippocampus (HIPP), ventral pallidum (VP), globus pallidus (GP), dorsolateral striatum (DSTr), NAc, VTA, bed nucleus of the stria terminalis (BNST) and central nucleus of the amygdala (CeA)[1, 2, 9]. In general, the expression patterns of EC biosynthetic enzymes (e.g. NAPE-PLD and DAGLα) and hydrolytic EC clearance enzymes (e.g. FAAH and MAGL) are similar to that for $CB_1$Rs across the regions depicted here[201, 202]. Within the amygdala, $CB_1$, DAGLα, MAGL and FAAH expression is highest in the lateral and basolateral nuclei, with substantially lesser expression in the central nucleus[56, 201]. In the dorsal striatum there is a comparable medial-lateral gradient of $CB_1$ and DAGLα expression with greater levels of expression evident in lateral aspects[201, 203]. Comparatively weaker $CB_1$, DAGLα and FAAH expression is observed in the NAc[201]. Although little to no $CB_1$ expression is found in dopamine cells in the NAc, DAGLα has been found in both dopaminergic and non-dopaminergic cells in this region[204].

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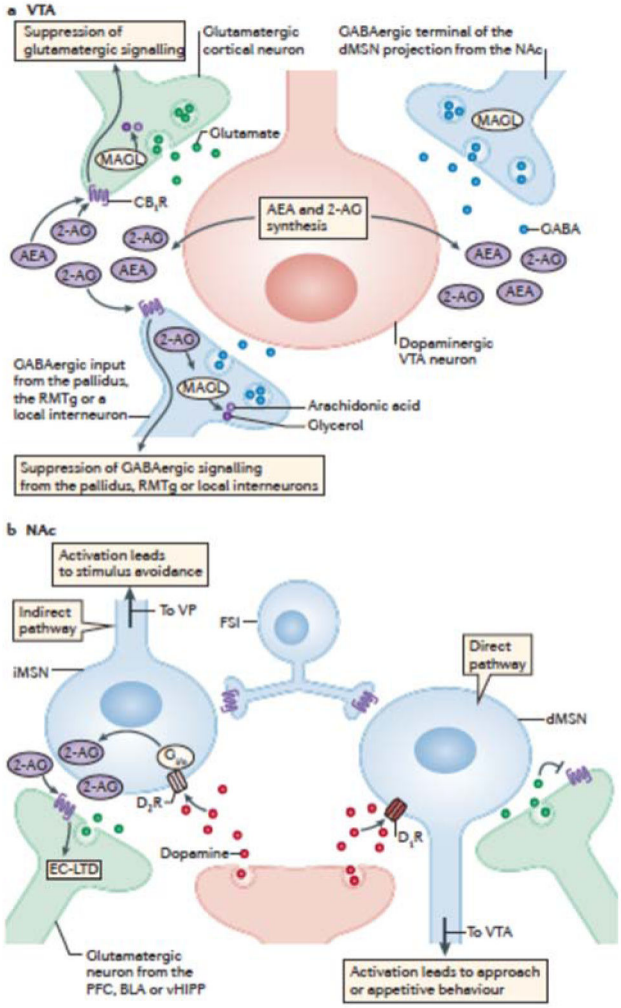


**Figure 3. Endocannabinoid influences in the VTA and NAc contributing to approach and avoidance behavior**

*a. EC influences on VTA synaptic signaling*. Endocannabinoids produced by dopaminergic VTA neurons act on $CB_1$Rs on nearby glutamatergic and GABAergic terminals before being degraded by ABHD6 or MAGL. $CB_1$Rs mediate robust inhibition of GABA inputs arising from the pallidus, RMTg nucleus and local interneurons onto VTA DA cells [205] and most evidence points to a role for 2-AG but not AEA in these processes[109, 206]. $CB_1$Rs are also localized on glutamatergic terminals synapsing on VTA DA neurons, with relatively greater expression on VGLUT1-positive terminals of cortical origin compared with VGLUT2-expressing terminals of subcortical origin[207]. Extensive evidence demonstrates EC-mediated suppression of glutamate signaling in VTA[208]. Thus, ECs play a prominent role in fine-tuning the activity of the mesolimbic DA projection through modulation of both excitatory and inhibitory signaling in the VTA. *b. EC influences on NAc synaptic signaling.* The majority of NAc neurons (>90%) are GABAergic medium spiny neurons (MSNs) that comprise the direct and indirect projection pathways. Direct pathway MSNs (dMSNs) project to midbrain regions including the VTA and activation of this pathway increases behavior toward a stimulus (approach or appetitive behavior). Indirect pathway MSNs

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(iMSNs) project to the ventral pallidum (VP) and activation of this pathway increases the stimulus avoidance[209]. dMSNs express excitatory $D_1$ DA receptors and iMSNs express inhibitory $D_2$ DA receptors, and thus reward-related phasic DA release activates the direct pathway and inhibits the indirect pathway, thereby increasing approach behavior and reducing avoidance behavior[210]. NAc MSN activity is also heavily modulated by glutamatergic inputs from the PFC, BLA and ventral hippocampus that express $CB_1$Rs[211], $CB_1$-mediated suppression of excitatory signaling (EC-LTD) is preferentially active at iMSN synapses[212] possibly resulting from $D_2$ receptor-mediated EC production from iMSN cell bodies[213]. Thus, increased NAc EC formation preferentially reduces excitatory input to iMSNs vs. dMSNs, resulting in decreased avoidance behavior. Through these mechanisms, increased EC signaling in the NAc increases approach behavior while reducing avoidance-related processing thereby enhancing appetitive responding toward a stimulus. $CB_1$Rs are also expressed on terminals of fast-spiking interneurons (FSIs) in the NAc, the majority of which are electrically and chemically coupled and provide direct innervation to adjacent MSNs[214]. FSIs exert important influence on the synchronization of neural ensemble activity and thus EC signaling may also exert critical influence on NAc output through feed-forward modulation of MSN network activity[214].

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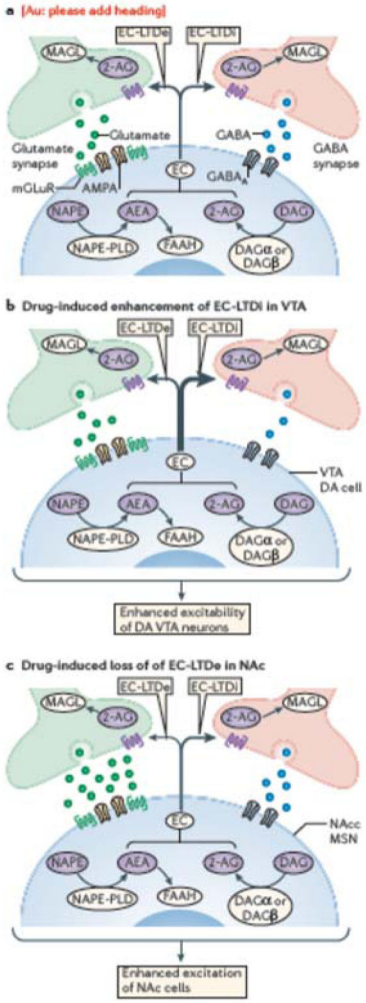


**Figure 4. Drug-induced alterations in EC-mediated synaptic plasticity**

Simplified summary of the effects of cocaine, Δ9-THC, and possibly other drugs of abuse on EC-mediated long-term depression (LTD). **a** | Under normal circumstances, EC-mediated long-term depression (EC-LTD) is induced by afferent stimulation with or without postsynaptic depolarization resulting in the 2-AG formation from postsynaptic cells. 2-AG activates $CB_1$Rs on stimulated or neighboring non-stimulated neurons, which together with other events (e.g. increased $[Ca^{+2}]$, NMDAR stimulation, DA $D_2$R stimulation, etc.) results in persistently decreased neurotransmitter release. The presynaptic signaling mechanisms contributing to EC-LTD are not fully understood. Depending on brain region, EC-LTD of both excitatory (glutamatergic) and inhibitory (GABAergic) afferents has been described. **b** | Repeated cocaine exposure facilitates the EC-LTD of inhibitory signaling in the VTA[109, 110] resulting in diminished inhibitory constraint of VTA DA cell activity and increased excitability. **c** | In contrast, EC-LTD of excitatory signaling is lost in the NAc medium spiny neurons (MSN) following exposure to either cocaine or Δ9-THC[108, 113, 114], resulting in diminished constraint of glutamatergic release and increased excitation of NAc cells. Thus, drug exposure results in concurrent loss of EC-mediated plasticity that normally provides inhibitory control over VTA DA cell excitation and loss of EC-mediated plasticity that

normally constrains excitatory signaling in the NAc terminal region, conferring an overall enhancement of mesolimbic signaling.

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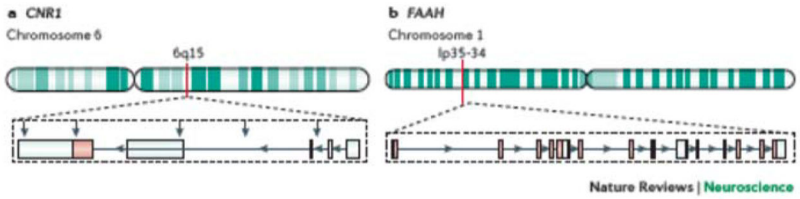


**Figure 5. CNR1 and FAAH genes and genetic variants associated with addiction**

The EC genes primarily studied to date in relation to genetic associations with addiction are *CNR1* and *FAAH*. The human *CNR1* gene, which encodes the $CB_1R$, maps to chromosome 6 specifically in the **cytogenetic band** 6q14-q15 and is transcribed from the minus strand (3′ to 5′ orientation) of the DNA. The gene contains four exons with the protein coding region located at the 5′ end of exon 4[146–148]. There are multiple mRNA variants of the *CNR1* with the prominent form encoding the canonical 472 aa protein. The *FAAH* gene is located on human chromosome 1, 1p35-34, and is transcribed from the plus strand (5′ to 3′ orientation). The gene contains 15 exons with functional protein domains encoded across multiple exons. Recently, another *FAAH* gene, *FAAH2*, was identified on chromosome X in cytogenetic band Xp11.21 that is composed of a 532 amino acids protein that share about 20% sequence identity with the canonical *FAAH1*[147]. There is evidence, though not all consistent, that genetic variants (red arrows) associated with addiction and related phenotypes such as reward sensitivity, impulsivity and negative affect are located within exon 4 (rs1049353), introns (rs2023239, rs1535255, rs806380) and the 3′UTR (AATn triplet repeat, rs806368) of *CNR1*[159146–148]. For the *FAAH* gene the polymorphic variant most associated with substance use disorders is rs324420 (exon 3)[166146, 147].

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## Table 1

Summary of $CB_1R$ and FAAH influence on non-cannabinoid drug reward.

| | EtOH | Nicotine | Opiates | Stimulants |
|---|---|---|---|---|
| **CB1 R KO** | ↓ CPP<br>↓ Self-Admin.<br>↓ EtOH-induced NAc DA | ↓ CPP<br>↓ Self-Admin. | ↓ CPP<br>↓ Self-Admin. | n/c CPP<br>n/c Self-Admin. |
| **CB1R Antagonist** | ↓ Self-Admin.<br>↓ Preference<br>↓ EtOH-induced NAc DA | ↓ CPP<br>↓ Self-Admin.<br>↓ NIC-induced NAc DA | ↓ CPP<br>↓ Self-Admin.<br>n/c MORPH-induced NAc DA | ↓ Self-Admin (AM251)<br>↓ COC effects on ICSS<br>n/c COC-induced NAc DA |
| **CB1R Agonist** | ↑ Self-Admin.<br>↑ Motivation for EtOH | ↑ CPP | ↑ CPP<br>↑ Motivation for heroin | ↓ Self-Admin<br>↓ COC effects on ICSS |
| **FAAH Inhibition** | ↑ Self-Admin.<br>↑ Preference | ↑ CPP (mouse, CB1R)<br>↓ CPP (rat, non-CB1R)<br>↓ Self-Admin (rat, non-CB1R)<br>↓ NIC-induced NAc DA (rat, non-CB1R) | n/c Self-Admin<br>n/c MORPH-induced VTA DA excitation | n/c COC-induced VTA DA excitation<br>n/c Self-Admin |

EtOH, ethanol; NIC, nicotine; MORPH, morphine; COC, cocaine; CPP, conditioned place preference; n/c. no change; Self-Admin., operant self-administration; Preference, relative consumption of EtOH vs. water; ICSS, intracranial self-stimulation (an index of brain reward function);