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Endocannabinoid signalling in reward and addiction

Loren H. Parsons¹ and Yasmin L. Hurd²

Abstract | Brain endocannabinoid (eCB) signalling 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 eCB signalling that may derive from pre-existing genetic factors or from prolonged drug exposure. Impaired eCB signalling contributes to dysregulated synaptic plasticity, increased stress responsivity, negative emotional states and cravings that propel addiction. Understanding the contributions of eCB disruptions to behavioural and physiological traits provides insight into the eCB influence on addiction vulnerability.

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.

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 (that is, positive reinforcement). The reward system is also essential for important negative hedonic responses, in which aversive or unpleasant events (for example, sickness or bodily harm) increase the likelihood of behaviours that will avoid or relieve these negative states (that is, negative reinforcement).

Seminal discoveries demonstrating that the effects of marijuana (*cannabis sativa*) are mediated by cannabinoid receptors in the brain propelled significant research initiatives that expanded our knowledge about the body's endogenous cannabinoid system (termed the endocannabinoid (eCB) system (ECS)), which is now acknowledged to have a prominent role in modulating brain reward function and maintaining emotional homeostasis. This Review examines the evidence for an eCB 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, which is characteristic of the addicted state. We explore the ECS signalling underlying these maladaptive processes and provide an overview of the existing literature regarding the genetic factors that are associated with the ECS to gain insight about the potential contribution of ECS signalling dysregulation to addiction disorders.

The ECS and reward circuits

The ECS comprises G protein-coupled receptors and small neuromodulatory lipid ligands, as well as biosynthetic and metabolic enzymes for the synthesis and degradation of the ligands, respectively. Two major types of cannabinoid receptor have been characterized and cloned: cannabinoid 1 receptors (CB1Rs; encoded by *CNR1*) and CB2Rs (encoded by *CNR2*). CB1Rs are the most-abundant G protein-coupled receptors that are expressed in the adult brain, and they show particularly dense expression in regions that have a known involvement in reward, addiction and cognitive function, including the amygdala, cingulate cortex, prefrontal cortex (PFC), ventral pallidum, caudate putamen, nucleus accumbens (NAc), ventral tegmental area (VTA) and lateral hypothalamus^{1,2}. CB2Rs are expressed mainly by immune cells, although recent evidence suggests that such receptors are also expressed in neurons, glia and endothelial cells in the brain³. CB1Rs and CB2Rs are coupled to similar transduction systems, primarily through G_i or G_o proteins. CB1Rs directly inhibit the release of GABA, glutamate and acetylcholine, which produce widespread effects on neural signalling across many neurotransmitter systems.

To date, the best-characterized eCB ligands are *N*-arachidonylethanolamide (anandamide (AEA)) and 2-arachidonoylglycerol (2-AG). Owing to their lipid nature, AEA and 2-AG are not stored in vesicles but are synthesized 'on demand' by cleavage of membrane precursors and immediate release through Ca²⁺-dependent mechanisms. AEA is derived from the phospholipid precursor *N*-arachidonoyl-phosphatidylethanolamine

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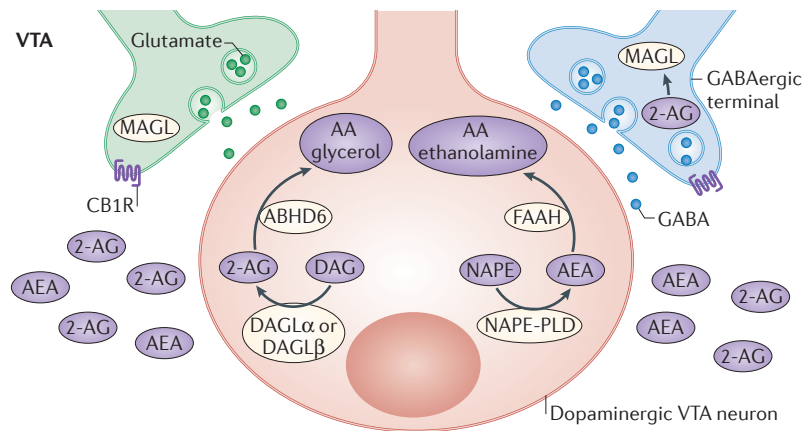


Figure 1 | Endocannabinoid biosynthesis, signalling and clearance. The most-commonly accepted route for *N*-arachidonylethanolamide (anandamide (AEA)) synthesis is from catalysis of *N*-arachidonoyl-phosphatidylethanolamine (NAPE) via a specific phospholipase D, *N*-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD). 2-arachidonylglycerol (2-AG) derives from the hydrolysis of 1,2-diacylglycerol (DAG) via the sn-1-selective DAG lipases (DAGLs) DAGLα and DAGLβ. DAGLα is found on the plasma membranes of both dopaminergic and non-dopaminergic neurons in the ventral tegmental area (VTA), opposite cannabinoid 1 receptor (CB1R)-expressing glutamate and GABA axon terminals²⁰⁰. Termination of endocannabinoid (eCB) signalling is initiated by cellular reuptake followed by enzyme-mediated hydrolytic cleavage. 2-AG hydrolysis is primarily mediated by presynaptic monoacylglycerol lipase (MAGL), although postsynaptic enzymes, including α,β-hydrolase 6 (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 postsynaptic organization of eCB biosynthetic and hydrolytic enzymes is generally conserved throughout the brain. AA, arachidonic acid.

(NAPE) and, although the precise mechanisms for AEA formation are not known, *N*-acyl-phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) is likely to have a role in this process. 2-AG derives primarily from the hydrolytic metabolism of 1,2-diacylglycerol (DAG) by the sn-1-selective DAG lipases (DAGLs) DAGLα and DAGLβ. AEA is primarily catabolized by fatty acid amide hydrolase 1 (FAAH1), and 2-AG is catabolized by monoacylglycerol lipase (MAGL) and, to a lesser extent, by α,β-hydrolase 6 (ABHD6), cyclooxygenase 2 (COX2) and FAAH1. The eCB catabolic enzymes have distinct cellular anatomical locations, with MAGL being localized predominantly in presynaptic terminals and FAAH1 being localized to the postsynaptic domain of neurons (FIG. 1). AEA and 2-AG both exert agonist activity at CB1R and CB2R. AEA binds with slightly higher affinity to CB1R than to CB2R 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 signalling on binding. 2-AG binds with essentially equal affinity at CB1R and CB2R 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), G protein-coupled receptor 55 (GPR55) and GPR119, and AEA exerts potent agonist effects at transient receptor potential (TRP) ion channels, including TRPV1.

Limbic system

A collection of brain structures that includes the amygdala, hippocampus, limbic cortex, limbic midbrain areas and anterior thalamic nuclei, regulates autonomic and endocrine function and participates in the control of emotion, motivation, long-term memory and olfaction.

Neurobiology of reward

Mesocorticolimbic dopamine (DA) pathways, which arise from the midbrain VTA, have a critical role in the mediation of reward. In particular, the VTA DA projection to the NAc (part of the ventral striatum) has a prominent role in positive reinforcement (FIG. 2), that is, the recognition of rewards in the environment and promotion of goal-directed behaviour (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 — increase NAc DA levels, and this neurochemical response contributes to subjective reward and positive reinforcement⁴. Components of the limbic system are also innervated by VTA DA neurons, including the amygdala, hippocampus, orbitofrontal cortex and parts of the PFC. These regions are interconnected in complex circuits that involve excitatory (primarily glutamatergic) and inhibitory (primarily GABAergic) projections⁵. 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 signalling in the dorsal striatum does not have a major influence in processing acute reward but has a key role in the development of compulsive forms of reward seeking and consumption⁶.

These same circuits participate in negative-reinforcement mechanisms that promote behaviours 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 signalling systems (such as corticotropin-releasing factor and dynorphin) and impaired function of anti-stress signalling systems (such as neuropeptide Y and nociceptin) in stress circuits that involve 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 heavily influenced by many other systems, including the cholinergic, opioid peptide, glutamatergic and GABAergic systems. CB1Rs are present in each of the interconnected structures involved in reward^{1,2,9}, where they exert widespread modulatory influences on excitatory and inhibitory signalling in a manner that influences reward processing^{10,11}. In particular, eCBs have a prominent role in

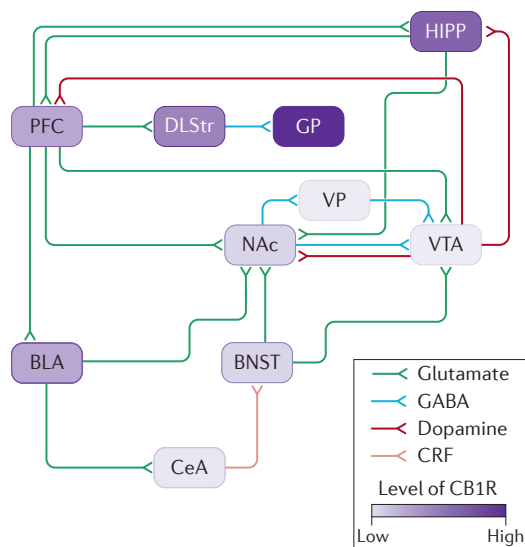


Figure 2 | Distribution of endocannabinoid signalling mechanisms within the brain reward circuits.

Cannabinoid 1 receptors (CB1Rs) are expressed throughout the regions implicated in reward and addiction, including the basolateral amygdala (BLA), prefrontal cortex (PFC), hippocampus (HIP), ventral pallidum (VP), globus pallidus (GP), dorsolateral striatum (DLStr), nucleus accumbens (NAc), ventral tegmental area (VTA), bed nucleus of the stria terminalis (BNST) and central nucleus of the amygdala (CeA)^{1,2,9}. In general, the expression patterns of endocannabinoid (eCB)-biosynthetic enzymes (for example, *N*-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD) and 1,2-diacylglycerol lipase- α (DAGL α)) and hydrolytic eCB-clearance enzymes (for example, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL)) are similar to those for CB1Rs across the regions depicted here^{201,202}. Within the amygdala, CB1R, DAGL α , MAGL and FAAH expression is highest in the lateral and basolateral nuclei, with substantially lesser expression in the CeA^{56,201}. In the dorsal striatum, there is a comparable medial-lateral gradient of CB1R and DAGL α expression, with greater levels of expression evident in lateral aspects^{201,203}. Comparatively weaker CB1R, DAGL α and FAAH expression is observed in the NAc²⁰¹. Although little to no CB1R expression is found in dopamine cells in the NAc, DAGL α has been found in both dopaminergic and non-dopaminergic cells in this region²⁰⁴. CRF, corticotropin-releasing factor.

fine-tuning the activity of the VTA–NAc DA projection and its influence on approach and avoidance behaviours that govern reward acquisition (FIG. 3).

eCB signalling and reward

Exogenous AEA and 2-AG both increase extracellular DA levels in the NAc in a CB1R-dependent manner¹², and the ECS exerts a strong influence on the fine-tuning of midbrain DA-cell activity¹³. Through these and other interactions, the ECS has a prominent influence on the hedonic effects of natural rewards, such as food¹⁴, sexual activity¹⁵ and social interaction¹⁶. This is mediated in part through a direct CB1R modulation of the mesolimbic DA response to natural reward¹⁶ and through the

interactions between the ECS and other signalling systems (such as those involving endogenous opioids and hypothalamic signalling molecules, among others)^{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. Δ^9 -THC is the primary psychoactive constituent in cannabis and exhibits low efficacy as an agonist at CB1R and CB2R¹⁹. In animal models, both Δ^9 -THC and synthetic CB1R agonists enhance brain reward function (as indexed by intracranial self-stimulation), produce rewarding effects in the paradigm of conditioned place preference (CPP) and are voluntarily self-administered (intravenously and also directly into the NAc shell and posterior VTA)²⁰. These effects are critically reliant on CB1R signalling and are highly dose-sensitive, with a rapid shift to negative-reinforcing effects with increasing dose.

In contrast to exogenous cannabinoid receptor agonists, pharmacological enhancement of eCB levels generally does not produce rewarding effects per se. For example, in most animal studies, selective eCB-clearance inhibitors do not support operant self-administration, do not produce CPP and do not alter brain stimulation reward thresholds in rats and mice²¹. Similarly, exogenously administered AEA or 2-AG, or selective FAAH or MAGL inhibitors, do not produce Δ^9 -THC-like discriminative stimulus effects. However, exogenous AEA and 2-AG both support operant self-administration in squirrel monkeys²¹ and produce rewarding and Δ^9 -THC-like effects in rats when they are administered after eCB-clearance inhibition^{9,22}. Concurrent FAAH and MAGL inhibition in mice produces Δ^9 -THC-like discriminative stimulus and behavioural effects^{22,23}. These findings suggest that robust engagement of eCB signalling 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, although this compound does not produce Δ^9 -THC- or nicotine-like discriminative stimulus effects and does not increase mesolimbic DA release²⁴. 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.

Cannabinoid receptor involvement in non-cannabinoid drug reward.

The presence of CB1Rs throughout brain reward circuits and the rewarding effects produced by CB1R activation allow for the possible influence of eCB signalling on the acute rewarding effects produced by non-cannabinoid substances (the effects of CB1R and FAAH manipulations on non-cannabinoid drug reward are summarized in TABLE 1). In general, drugs that activate CB1Rs do indeed seem to facilitate the rewarding effects of non-cannabinoid drugs. CB1R agonists increase the motivational and reinforcing effects of alcohol, nicotine and opiates, as indexed by animal models of drug reward (including the CPP and operant self-administration assays), whereas diminished

Intracranial self-stimulation

An operant behavioural paradigm in which subjects produce a behavioural response (such as a lever press or wheel turn) to receive brief electrical pulses into specific regions in the brain reward pathways.

Conditioned place preference (CPP).

A behavioural paradigm used to study the rewarding and aversive effects of drugs through Pavlovian conditioning.

Self-administered

In a medical sense, when a pharmacological substance is purposefully delivered by test subjects to themselves. Operant self-administration is a behavioural procedure in which experimental subjects learn to produce an operant response (for example, a lever press or nose poke) to receive a drug reward (such as an intravenous infusion, a small bolus for oral consumption or delivery of a discrete bolus of vapour that is inhaled).

Discriminative stimulus

A stimulus in a drug-discrimination paradigm that the animal has learned to associate with a predictable consequence (whether rewarding or unrewarding) and that increases the elicitation of a specific behaviour by the animal.

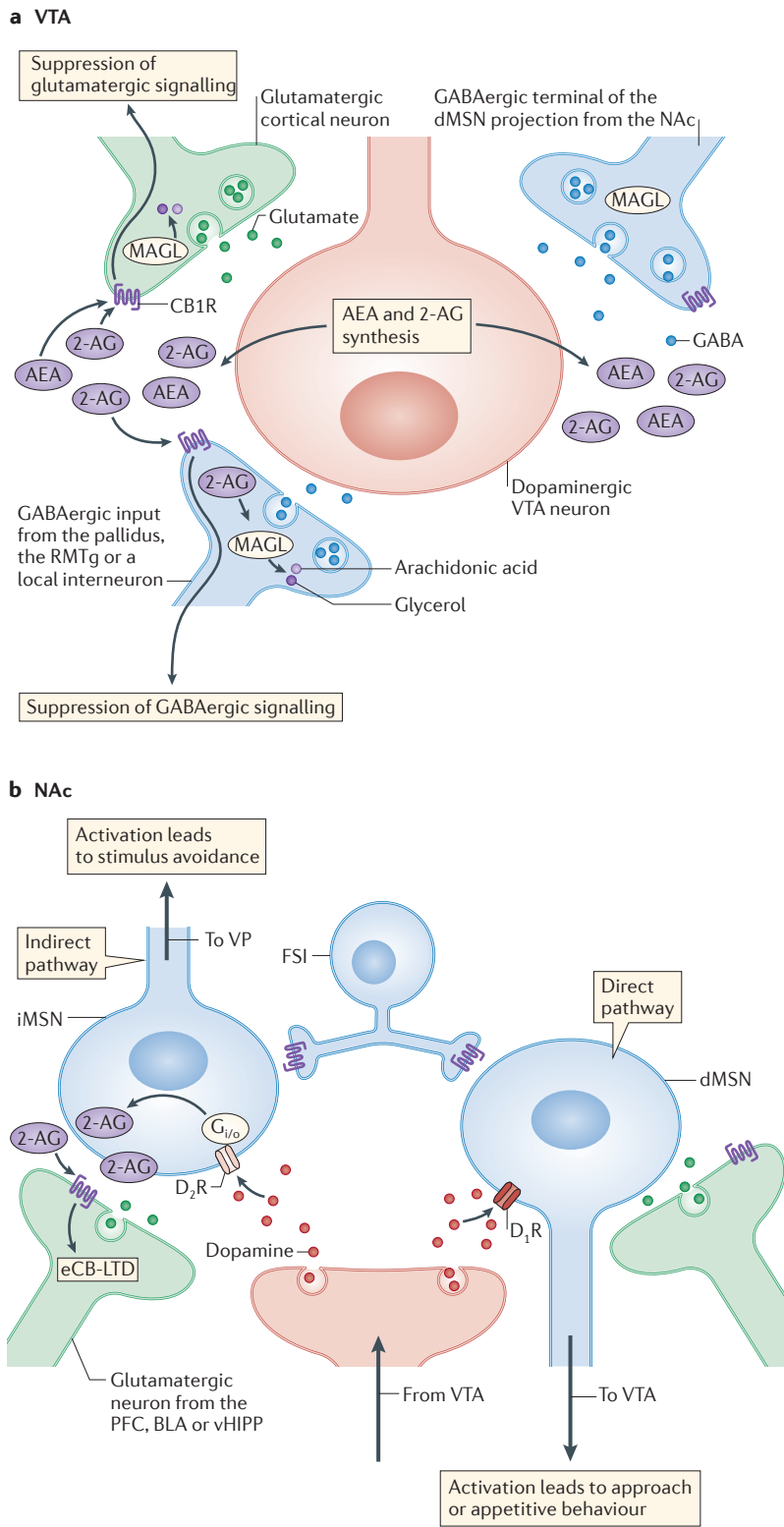


Figure 3 | Endocannabinoid influences in the VTA and NAc contributing to approach and avoidance behaviours. **a** | Endocannabinoids (eCBs) influence ventral tegmental area (VTA) synaptic signalling. 2-Arachidonoylglycerol (2-AG) produced by dopaminergic VTA neurons acts on cannabinoid 1 receptors (CB1Rs) on nearby glutamatergic and GABAergic terminals before being degraded by α,β -hydrolase 6 (ABHD6) or monoacylglycerol lipase (MAGL). CB1Rs mediate robust inhibition of GABA inputs arising from the pallidus, rostromedial tegmental (RMTg) nucleus and local interneurons onto VTA dopamine (DA) cells²⁰⁵, and most evidence points to a role for 2-AG but not *N*-arachidonyl ethanolamide (anandamide (AEA)) in these processes^{109,206}. CB1Rs are also localized on glutamatergic terminals synapsing on VTA DA neurons, with relatively greater expression on vesicular glutamate transporter 1 (VGLUT1)-positive terminals of cortical origin compared with VGLUT2-expressing terminals of subcortical origin²⁰⁷. Extensive evidence demonstrates eCB-mediated suppression of glutamate signalling in the VTA²⁰⁸. Thus, eCBs have a prominent role in fine-tuning the activity of the mesolimbic DA projection through modulation of both excitatory and inhibitory signalling in the VTA. **b** | The eCBs also influence nucleus accumbens (NAc) synaptic signalling. 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 behaviour towards a stimulus (approach or appetitive behaviour). Indirect-pathway MSNs (iMSNs) project to the ventral pallidum (VP), and activation of this pathway increases stimulus avoidance²⁰⁹. dMSNs express excitatory D₁ DA receptors (D₁Rs), whereas iMSNs express inhibitory D₂Rs; thus, reward-related phasic DA release activates the direct pathway and inhibits the indirect pathway, thereby increasing approach behaviour and reducing avoidance behaviour²¹⁰. NAc MSN activity is also heavily modulated by glutamatergic inputs from the prefrontal cortex (PFC), basolateral amygdala (BLA) and ventral hippocampus (vHIPP), which express CB1Rs²¹¹. CB1R-mediated suppression of excitatory signalling (eCB-mediated long-term depression (eCB-LTD)) is preferentially active at iMSN synapses²¹², possibly resulting from D₂R-mediated eCB production from iMSN cell bodies²¹³. Thus, increased NAc eCB formation preferentially reduces excitatory input to iMSNs versus dMSNs, resulting in decreased avoidance behaviour. Through these mechanisms, increased eCB signalling in the NAc increases approach behaviour while reducing avoidance-related processing, thereby enhancing appetitive responses towards a stimulus. CB1Rs 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 MSNs²¹⁴. FSIs exert important influence on the synchronization of neural ensemble activity and thus eCB signalling may also exert critical influence on NAc output through feedforward modulation of MSN network activity²¹⁴.

CB1R signalling (through either genetic deletion or pharmacological antagonism) attenuates the motivational and rewarding effects of these drugs^{11,25,26}. The effects of CB1R antagonism on alcohol and nicotine reward result in part from a diminished ability of these drugs to increase NAc DA release²⁷. Blockade of CB1Rs

specifically in the VTA decreases alcohol and nicotine self-administration^{28,29}, and blockade of CB1Rs specifically in the NAc reduces alcohol consumption^{28,30}. However, whereas nicotine reward is critically dependent on the mesolimbic DA system³¹, the motivational and rewarding effects of alcohol and opiates are less

Table 1 | Summary of CB1R and FAAH influences on non-cannabinoid drug reward

Genetic or pharmacological manipulation	Drug			
	Ethanol	Nicotine	Opiates	Stimulants
CB1R knockout	↓ CPP ↓ Operant self-administration ↓ Ethanol-induced NAc DA	↓ CPP ↓ Operant self-administration	↓ CPP ↓ Operant self-administration	• No change in CPP • No change in operant self-administration
CB1R antagonist	↓ Operant self-administration ↓ Preference ↓ Ethanol-induced NAc DA	↓ CPP ↓ Operant self-administration ↓ Nicotine-induced NAc DA	↓ CPP ↓ Operant self-administration • No change in morphine-induced NAc DA	↓ Operant self-administration (AM251) ↓ Cocaine effects on ICSS • No change in cocaine-induced NAc DA
CB1R agonist	↑ Operant self-administration ↑ Motivation for ethanol	↑ CPP	↑ CPP ↑ Motivation for heroin	↓ Operant self-administration ↓ Cocaine effects on ICSS
FAAH inhibition	↑ Operant self-administration ↑ Preference for ethanol versus water	↑ CPP (mouse, CB1R) ↓ CPP (rat, non-CB1R) ↓ Operant self-administration (rat, non-CB1R) ↓ Nicotine-induced NAc DA (rat, non-CB1R)	• No change in operant self-administration • No change in morphine-induced VTA DA excitation	• No change in cocaine-induced VTA DA excitation • No change in operant self-administration

CB1R, cannabinoid 1 receptor; CPP, conditioned place preference; DA, dopamine; FAAH, fatty acid amide hydrolase; ICSS, intracranial self-stimulation (an index of brain reward function); NAc, nucleus accumbens; VTA, ventral tegmental area.

DA-dependent^{32,33}, and the CB1R modulation of the rewarding effects of these drugs probably involves non-dopaminergic mechanisms. Indeed, CB1R antagonism does not alter opiate-induced increases in NAc DA levels but reduces opiate reward through the prevention of opiate-induced reductions in ventral pallidal GABA release³⁴. In comparison to these drugs, CB1R manipulations on psychostimulant reward have modest and less-consistent effects. CB1R agonists reduce the facilitation of brain stimulation reward produced by cocaine and reduce cocaine self-administration^{35,36}. Most reports indicate that CB1R antagonism does not affect psychostimulant reward (as assessed by cocaine-induced enhancement of brain stimulation reward, CPP and self-administration) or cocaine-induced increases in NAc DA levels³⁷ (but see REFS 27,37).

Recent evidence in mice also implicates CB2Rs 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 that these findings are influenced by species differences in *CNR2* splicing that confer distinct CB2R structure, function or pharmacology³⁹.

Alterations in brain eCB levels elicited by drugs of abuse.

Given the on-demand nature of eCB production and the associated modest eCB signalling tone under baseline conditions, the robust influence of cannabinoid receptor signalling on non-cannabinoid drug reward has led to the hypothesis that drug exposure increases brain eCB formation. Substantial evidence demonstrates that there are alcohol-induced alterations in post-mortem eCB content in the rodent brain, although inconsistencies among studies cloud definitive conclusions regarding the direction of change and the regional nature of the effects⁴⁴. 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 than after non-contingent alcohol exposure^{28,30}. By contrast, extracellular AEA levels in the NAc are unaltered by alcohol self-administration and are decreased by non-contingent alcohol administration. Alcohol also seems to induce region-specific changes in brain-tissue eCB levels, with alcohol-induced disruptions being consistently observed in striatal regions^{30,45,46} but not in frontal cortical areas²⁸. This is consistent with evidence that alcohol consumption is reduced by CB1R antagonism in the VTA and NAc but not in the PFC^{28,30,47}.

Similarly to alcohol, nicotine alters eCB levels in the 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 tissue⁴⁸. Intravenous nicotine self-administration increases extracellular levels of both AEA and 2-AG in the rat VTA, and the effect on 2-AG is sensitized by chronic nicotine exposure⁴⁹. Interestingly, although VTA 2-AG levels are elevated by both voluntary and non-contingent nicotine exposure, VTA AEA levels are increased only by voluntary nicotine self-administration⁴⁹. Together with the evidence of distinct patterns of brain eCB levels induced by volitional versus non-contingent alcohol exposure^{28,30}, these data suggest that brain eCB 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 eCB 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

Non-contingent
Drug delivery that is involuntary (experimenter-administered) or is not dependent on a behavioural response by an experimental subject; sometimes referred to as forced administration.

rat NAc³⁰. Psychostimulants generally produce modest disruptions in brain eCB content, with subtle increases and decreases in 2-AG concentration in forebrain following non-contingent acute and chronic cocaine exposure, respectively (no other alterations are evident regardless of region analysed)^{48,52}. Moreover, voluntary cocaine self-administration does not alter rat extracellular NAc eCB levels³⁰ but decreases 2-AG content in frontal cortex and hippocampal tissue^{53–55}.

Collectively, these findings indicate that alcohol, nicotine and opiates alter brain eCB content, consonant with the CB1R influence on the behavioural effects produced by these drugs. The generally modest effects of psychostimulants on brain eCB levels are in-line with the subtle CB1R influence on psychostimulant-induced behaviours. Similarly to that seen with multiple biological conditions, drug exposure often produces distinct and sometimes opposite effects on brain AEA and 2-AG levels. This suggests differential regulation of the synthesis and/or degradation of these eCB moieties at specific synapses that may arise from the segregation of MAGL and FAAH in the pre- and postsynaptic compartments⁵⁶, or the hypothesized role of AEA and 2-AG in regulating 'tonic' and 'phasic' signalling in the ECS, respectively⁵⁷. Although a general picture of drug-induced alterations in brain eCB levels is emerging, experimental differences between studies — including the drug dose used, the method of drug exposure and the duration of treatment — make it difficult to draw strong conclusions, and additional studies are warranted.

Influence of eCB tone on drug reward. eCBs are rapidly degraded, so strategies that reduce eCB clearance have been used as a means to further investigate the eCB influence on drug reward. Most investigations have focused on the effects of FAAH inhibition, because selective tools for inhibiting MAGL and other eCB-clearance enzymes were not available until recently. Such studies have shed light on important species differences that confound 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}, but FAAH inhibition in rats prevents nicotine-induced CPP, diminishes nicotine self-administration and blunts nicotine-induced increases in NAc DA release⁶⁰. The potentiation of nicotine reward in mice by FAAH inhibition is CB1R-mediated, whereas the reduction in nicotine reward in rats results from activation of PPAR α by non-cannabinoid lipids, such as oleoylethanolamide and palmitoylethanolamide, that are hydrolytically cleared by FAAH⁶¹. FAAH inhibition also produces distinct species-related alterations in alcohol consumption, with increased intake being observed in mice but not in 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, 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 activity⁶⁸, and this may contribute to enhanced sensitization of both cocaine-induced motor activity and mesolimbic DA responses following repeated cocaine exposure⁶⁹. Other studies have investigated the effects of putative eCB 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^{70–73}.

Although growing evidence implicates ECS influences in the modulation of acute drug reward, additional efforts are needed to further clarify the nature of eCB disruptions caused by different classes of abused drug and the neural mechanisms through which these eCB influences are mediated. Selective inhibitors of 2-AG clearance have recently been developed, but studies using them are still in their infancy and 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 signalling and plasticity related to both drug and natural rewards. Nevertheless, the role of the CB1R in drug reward is unequivocal and, although there is evident complexity related to the effects produced by eCB-clearance inhibition (producing discrete modulation of eCB tone in specific synapses and circuits when compared with broad CB1R activation by exogenous CB1R agonists), the extant evidence strongly supports an eCB influence on the sensitivity to, and motivation for, several drugs of abuse.

eCB signalling in addiction

Numerous factors influence the transition from intermittent, controlled drug use to the compulsive forms of drug-seeking and drug-taking behaviour that characterize addiction. Substantial evidence implicates genetic influences in the development of substance-use disorders (SUDs) and pathological forms of eating, sexual behaviour and gambling⁷⁴, and it is increasingly recognized that epigenetic mechanisms drive lasting changes in addiction-related gene expression⁷⁵. Long-term drug exposure induces lasting neuroadaptations in motivational mechanisms that propel drug-seeking behaviour and drug 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 (for example, dysphoria, anxiety and irritability) emerge during abstinence. These negative emotional states arise from the recruitment of stress signalling 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 amygdala⁵. Renewed drug consumption alleviates these negative affective states, and this is

Epigenetic mechanisms

Methods by which functionally relevant changes to the genome occur that do not involve disruptions in the nucleotide sequence of DNA; these include DNA methylation, histone modification and non-coding RNA-associated gene silencing

Extended amygdala

A grouping of brain regions that orchestrate emotional behavioural responses and includes the central nucleus of the amygdala, subnucleus of the amygdala, subnucleus of the amygdala, nucleus accumbens shell and the bed nucleus of the stria terminalis.

Conditioned reinforcement

The process through which neutral stimuli acquire motivational properties through association with a primary reinforcer.

Stochastic optical reconstruction microscopy

A super-resolution imaging technique that uses sequential activation and time-resolved localization of photoswitchable fluorophores to create high-resolution images enabling precise fluorophore localization with nanometre resolution.

conceptualized to motivate compulsive drug use through negative reinforcement⁵. 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 rewards⁶.

eCBs exert prominent modulatory influence on 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 eCB function participate in the development and maintenance of addictions, including pathological forms of eating (BOX 1). The following sections consider the consequences of chronic drug exposure on eCB signalling within the reward circuitry and related disruptions in synaptic plasticity, affective state and learning and memory mechanisms related to extinction and relapse. Finally, evidence is discussed for an influence of innate disruptions in ECS function (eCB gene polymorphisms) as vulnerability factors for substance abuse and addictive disorders in humans.

Chronic drug exposure and eCB 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, one study⁷⁶ reported that there was downregulation of brain CB1Rs in daily cannabis users, that the

level of downregulation correlated with the number of years of cannabis use and that this downregulation was reversed after 1 month of monitored abstinence. Another PET study reported a global reduction in CB1R availability⁷⁷ driven by differences in the temporal lobe, anterior and posterior cingulate cortex and NAC. Similarly, animals given non-contingent chronic cannabinoid exposure exhibit decreased CB1R function throughout the brain^{78,79}. Recent experiments using stochastic optical reconstruction microscopy demonstrate that chronic exposure to clinically relevant doses of Δ^9 -THC results in a startling loss of CB1Rs on terminals of perisomatically projecting GABAergic interneurons in the mouse hippocampus and internalization of the remaining CB1Rs⁸⁰. The resulting deficits in inhibitory CB1R control over hippocampal GABA release persisted during several weeks of Δ^9 -THC abstinence, and this may underlie the enduring loss of hippocampal long-term potentiation (LTP) in rodents and memory deficits in humans evident following chronic cannabinoid exposure⁸¹.

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^{82,83}, and frequent cannabis smokers present decreased AEA and increased 2-AG levels in blood^{84,85}, although increased serum AEA levels are evident following at least 6 months of cannabis abstinence⁸⁶. The contribution of these disruptions to cannabis-use disorder and related physiological and behavioural disruptions is presently unexplored. However, as discussed below, eCBs provide important homeostatic constraint over emotional state⁸⁷ and sleep function⁸⁸, and it is conceivable that Δ^9 -THC-induced impairment of eCB signalling contributes to the negative emotional states and sleep disturbances present during protracted cannabis abstinence^{89,90}.

Several findings support the hypothesis that chronic exposure to non-cannabinoid drugs disrupts eCB signalling and processing. Chronic alcohol exposure in rodents alters eCB-related gene expression in a manner sensitive to the intermittent nature of alcohol exposure and post-alcohol abstinence period⁹¹ and downregulates CB1R expression and function^{45,92}. Post-mortem studies of alcohol-dependent humans also demonstrate disrupted CB1R expression in the ventral striatum and cortical regions⁹³, and *in vivo* imaging studies demonstrate decreased CB1R availability in heavy-drinking alcoholics that persists for at least 1 month of abstinence^{94,95} (but see REF. 96). Although a potential contribution of variants of *CNR1* (which encodes CB1R) to these observations cannot be excluded, a common interpretation based on animal studies is that these CB1R adaptations in humans with alcoholism are a consequence of prolonged alcohol-induced increases in brain eCB levels. This is supported by evidence of transient recovery (and perhaps eventual upregulation) of CB1R function in humans during protracted alcohol abstinence^{92,97}.

In rodents, chronic nicotine exposure induces distinct age-related disruptions in CB1R binding, with increased levels being evident in the PFC, VTA and hippocampus

Box 1 | Endocannabinoid signalling and eating disorders

Food and drug addiction derive in part from aberrant brain reward function and share overlapping neuroadaptations in the mesolimbic system¹⁷⁶. Similarly 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 endocannabinoid (eCB) function¹⁷⁷. Anorexia nervosa and binge-eating disorder are associated with increased blood *N*-arachidonylethanolamide (anandamide (AEA)) levels and diminished levels of leptin¹⁷⁸ (an anorectic hormone that inhibits AEA synthesis). Anorexia is associated with a *CNR1* (the gene encoding cannabinoid 1 receptor (CB1R)) AAT-triplet repeat ((AAT)_n) polymorphism¹⁷⁹ (but see REF. 180) and a synergistic association between the C385A fatty acid amide hydrolase (*FAAH*) and *CNR1* rs1049353 polymorphisms¹⁸¹. The C385A *FAAH* polymorphism is also associated with obesity¹⁸², although it is unknown whether this may influence hedonic mechanisms, metabolism or both. Anorexia and bulimia nervosa are also associated with elevated plasma *CNR1* mRNA levels¹⁸³, increased CB1R availability in frontal cortical areas¹⁸⁴ and a nonsynonymous *CNR2* (the gene encoding CB2R) polymorphism¹⁸⁵. Preclinical models of anorexia suggest therapeutic effects for Δ^9 -tetrahydrocannabinol (Δ^9 -THC)¹⁸⁶ (but see REF. 187). Although two small clinical trials did not show Δ^9 -THC efficacy¹⁷⁶, a larger trial demonstrated small but significant weight gain in women with severe anorexia nervosa following 4 weeks of treatment with dronabinol¹⁸⁸. Conversely, in binge-eating disorder, the CB1R 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}, with symptoms including increased anxiety and excessive consumption on renewed access to palatable food. These symptoms result from increased corticotropin-releasing factor 1 (CRF1; also known as CRHR1) signalling in the central nucleus of the amygdala (CeA)⁸ and are reversed by increased CeA 2-arachidonoylglycerol (2-AG)–CB1R signalling¹⁹³. This suggests that dysregulated CeA function is a common factor contributing to pathological motivation for drugs and food, and that CeA eCB signalling may counter withdrawal-related stress signalling.

of adolescent, but not adult, rats⁹⁸ and increased hippocampal and decreased striatal CB1R binding being seen in adult rats during protracted nicotine abstinence⁹⁹. Few studies have investigated altered CB1R binding following chronic opiate or psychostimulant exposure, but findings in rodents implicate impaired CB1R function in the development and expression of opiate dependence^{100,101} and demonstrate that chronic cocaine use increases CB1R binding in the dorsal striatum, NAc and cortical areas¹⁰². Interestingly, detoxified cocaine addicts present significant increases in plasma AEA and decreases in plasma 2-AG content¹⁰³, but the functional consequence of these disturbances is not known. Overall, accruing data suggest that long-term exposure to various drug classes compromises eCB processing and CB1R expression and function. As discussed below, these perturbations may contribute to aberrant neural signalling 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. eCB signalling at CB1Rs is implicated in several forms of synaptic plasticity, most commonly in depolarization-induced suppression of excitatory transmission (DSE) or inhibitory transmission (DSI), short-term depression (STD) and long-term depression (LTD; a prolonged form of weakened synaptic strength)^{68,104}. STD, DSE and DSI are mediated primarily by 2-AG signalling, 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 nigra¹⁰. By comparison, eCB-mediated LTD can persist for hours or 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 striatum¹⁰.

Acute and chronic alcohol exposure reduces CB1R-dependent plasticity, resulting in long-lasting disinhibition of striatal output neurons and diminished eCB-mediated LTD (eCB-LTD) at inhibitory striatal synapses^{105,106}. Because the dorsal striatum mediates reward-guided learning and habitual behaviour, these eCB disruptions may contribute to maladaptive habitual behaviour that perpetuates addiction¹⁰⁷. Cocaine diminishes eCB-LTD of excitatory transmission in the NAc¹⁰⁸ and facilitates eCB-LTD of inhibitory signalling at VTA DA synapses^{109,110}, resulting in diminished inhibitory control over VTA DA-cell activity and heightened excitatory signalling in the NAc (FIG. 4). Cocaine also disrupts eCB-LTD of excitatory transmission in the BNST¹¹¹, 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 behaviour. Similarly, chronic Δ^9 -THC or synthetic CB1R agonist exposure abolishes eCB-LTD of excitatory and inhibitory signalling in the NAc and hippocampus^{113,114}, which may significantly affect

reward processing mediated by these regions. Little is known regarding opiate- or nicotine-induced disruptions in eCB-mediated synaptic plasticity, although cue-induced reinstatement of nicotine-seeking behaviour (an animal model of relapse) relies in part on the induction of CB1R-mediated LTP of cortical synapses in the BNST¹¹⁵. Thus, chronic drug exposure disrupts eCB-mediated forms of synaptic plasticity in several regions involved in reward processing. As discussed below, impaired eCB-mediated plasticity may also contribute to dependence-related affective disruptions that serve to sustain drug dependence.

Withdrawal-related affective disruption. Stress has a prominent role in the development of addiction¹¹⁶, and stress exposure disrupts eCB-mediated plasticity in regions that participate in emotional control, including the NAc, amygdala and BNST¹¹⁷. 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 pre-existing 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 eCB-mediated forms of synaptic plasticity, and deficient eCB signalling is associated with increased anxiety and depression. As such, impaired eCB function may contribute to the 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 CB1Rs exhibit greater anxiety-like behaviour than normal animals during nicotine withdrawal¹⁹, although innate anxiety-like behaviour in the knockout mice clouds interpretations. Studies evaluating eCB-clearance inhibition provide more-direct insight into withdrawal-related eCB disruption and negative affect. Acute FAAH inhibition reverses enhanced anxiety-like behaviour that is normally present during both nicotine and alcohol withdrawal^{65,120}, and the eCB-transport inhibitor AM404 attenuates depression-like behaviour during nicotine withdrawal¹²¹. Post-traumatic stress disorder is particularly prevalent among individuals with alcohol-use disorders, and this is often modelled 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}. In addition, acute FAAH inhibition by LY2183240 reduces fear-potentiated startle in high alcohol-preferring, but not low alcohol-preferring, mice¹²⁴, consistent with the efficacy of FAAH inhibition for accelerating the extinction of aversive memory¹²⁵. LY2183240 also enhances the conditioned rewarding effects of alcohol without altering alcohol consumption itself, suggesting that FAAH

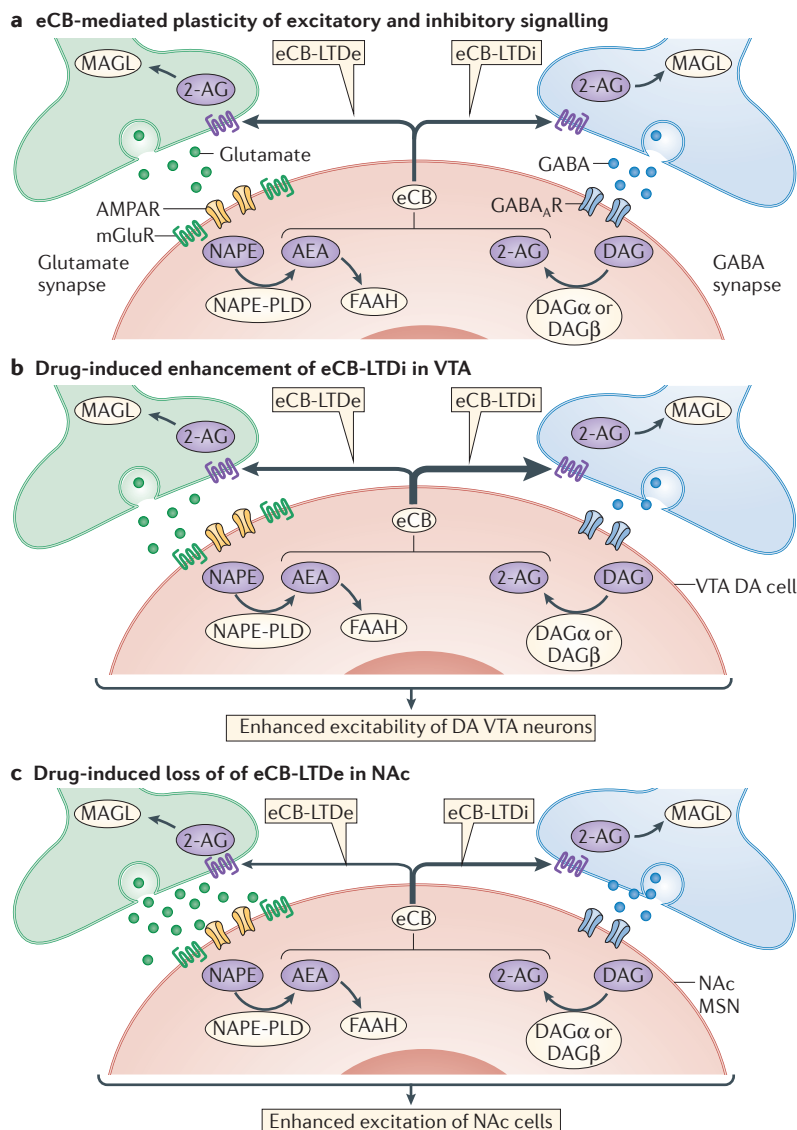


Figure 4 | Drug-induced alterations in endocannabinoid-mediated synaptic plasticity. Simplified summary of the effects of cocaine, Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and possibly other drugs of abuse on endocannabinoid-mediated long-term depression (eCB-LTD). **a** | Under normal circumstances, eCB-LTD is induced by afferent stimulation with or without postsynaptic depolarization, resulting in 2-arachidonoylglycerol (2-AG) formation from postsynaptic cells. 2-AG activates cannabinoid 1 receptors (CB1Rs) on stimulated or neighbouring, non-stimulated neurons, which together with other events (for example, increased $[Ca^{2+}]$, NMDA receptor (NMDAR) stimulation and dopamine (DA) D2 receptor stimulation) results in persistently decreased neurotransmitter release. The presynaptic signalling mechanisms contributing to eCB-LTD are not fully understood. Depending on brain region, eCB-LTD of both excitatory (glutamatergic; eCB-LTDe) and inhibitory (GABAergic; eCB-LTDi) afferents has been described. **b** | Repeated cocaine exposure facilitates eCB-LTDi in the ventral tegmental area (VTA)^{109,110}, resulting in diminished inhibitory constraint of VTA DA-cell activity and increased excitability. **c** | By contrast, eCB-LTDe is lost in the nucleus accumbens (NAc) medium spiny neurons (MSN) following exposure to either cocaine or Δ^9 -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 eCB-mediated plasticity that normally provides inhibitory control over VTA DA-cell excitation and that normally constrains excitatory signalling in the NAc terminal region, conferring an overall enhancement of mesolimbic signalling. AEA, N-arachidonylethanolamide (anandamide); AMPAR, AMPA receptor; DAGL, 1,2-diacylglycerol lipase; FAAH, fatty acid amide hydrolase; GABA_AR, GABA_A receptor; mGluR, metabotropic glutamate receptor; NAPE, N-arachidonoyl-phosphatidylethanolamine; NAPE-PLD, N-acyl-phosphatidylethanolamine-specific phospholipase D.

inhibition influences memory-related processes (conditioned fear and conditioned alcohol reward) in animals predisposed towards high alcohol consumption.

Addiction-related learning and memory

Both positive and negative memories and conditioned cues associated with drug use perpetuate drug-seeking behaviour and the continued cycle of abuse. The ECS has a prominent role in learning and memory processes¹²⁶, and CB1R signalling 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 CB1R 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.

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 abstinence¹²⁷. In addition to conditioned drug memories, acute exposure to a preferred drug or pharmacologically related agent (that is, drug priming) and stressful events can precipitate relapse¹¹⁶.

Animal models of relapse demonstrate an important cannabinoid influence on the reinstatement of extinguished drug-seeking and drug-taking behaviours. Δ^9 -THC and synthetic CB1R agonists reinstate drug-seeking for cannabinoids, alcohol, nicotine, opiates and cocaine, whereas CB1R antagonists attenuate drug-seeking behaviour associated with each of these drugs^{25,128,129}. CB1Rs in the PFC and NAc shell influence cue-induced reinstatement of both heroin- and nicotine-seeking behaviour, whereas CB1Rs in the basolateral amygdala contribute to cue-induced nicotine- but not heroin-seeking behaviour^{130,131}. Despite the subtle effects of CB1R inactivation on psychostimulant self-administration, CB1R antagonism attenuates drug-primed, cue-induced and some forms of stress-induced reinstatement of cocaine- and methamphetamine-seeking behaviour in

rats²⁵. Thus, CB1R signalling modulates drug-seeking for various pharmacologically distinct drugs. There is also evidence that CB1R antagonism blocks both cue- and priming-induced reinstatement of seeking behaviour for non-drug rewards, such as sucrose and corn oil^{132,133} (but see REF. 134). Accordingly, CB1R signalling seems to participate in the modulation of conditioned reward in general.

Drug-primed and cue-induced nicotine- and cocaine-seeking behaviour are reduced following acute FAAH inhibition that leads to elevated AEA levels^{25,60}, which may be surprising considering that CB1R agonists enhance both nicotine- and cocaine-seeking behaviour²⁵. However, inhibition of eCB clearance probably amplifies eCB signalling preferentially in circuits or synapses activated by a given stimulus (in this case, drug-seeking behaviour), rather than inducing more-widespread indiscriminate CB1R activation, as produced by exogenous CB1R agonists. Moreover, FAAH hydrolyses a large range of fatty acid moieties, and the effects of FAAH inhibition on drug-seeking behaviour may involve non-cannabinoid lipid signalling. In this regard, it is notable that the eCB transport inhibitor VDM11 attenuates both nicotine- and cue-induced nicotine-seeking behaviour⁷¹, and this compound may preferentially block AEA clearance with weaker effects on non-cannabinoid lipids^{135,136}. Similarly, the eCB transport inhibitor AM404 dose-dependently attenuates nicotine- and cue-induced nicotine-seeking behaviour without altering nicotine self-administration⁷².

In contrast to nicotine- and cocaine-seeking behaviours, neither FAAH inhibition nor eCB transport inhibition alter cue- or stress-induced reinstatement of alcohol-seeking behaviour^{65,137}. However, studies in humans with alcoholism suggest a relationship between eCB 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 craving¹³⁸. However, recently detoxified individuals with alcoholism present significantly lower baseline plasma AEA levels than non-dependent social drinkers and, although alcohol-related cues elicit more-intense cravings in alcoholics, these individuals do not present significant cue-induced increases in plasma AEA. This blunted AEA response may reflect aberrant eCB processing in people with alcoholism, but further investigations are needed to confirm a direct link between this potential peripheral biomarker and brain eCB signalling, as well as possible causal relationships between dysregulated eCB processing and behaviour.

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 (for example, relapse)¹²⁷. One approach for reducing the motivational impact of drug-associated cues is through extinction training, in which a subject learns that these cues no longer have predictive value. However, extinction therapy is generally ineffective

for reducing relapse in both humans¹³⁹ and rodents¹⁴⁰, and it is conceivable this is a consequence of diminished learning mechanisms required to override the original cue-association memory. The ECS has a prominent role in memory extinction, and deficient CB1R signalling 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 consumption¹²⁴. Because aversive memory may be involved in relapse to drug taking¹⁴³, deficient eCB signalling following long-term drug exposure may contribute to the limited efficacy of extinction therapy for addiction.

eCB gene polymorphisms and addiction

Approaches to explore the contribution of the ECS to addiction disorders in humans often involve heritability considerations, as it is now acknowledged that genetics plays an important part in drug addiction vulnerability, accounting ~30–80% for risk depending on the drug class^{144,145}. Based on the growing evidence of a role for the ECS in regulating reward, mood and cognition and owing 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. Similarly to the preclinical animal studies described above, most investigations have focused on *CNR1* and *FAAH*^{146,147}. Consistent with most genetic investigations, important confounding factors include race, ethnicity, type of drug, polysubstance use and population sample size. Nevertheless, although they are not all equivocal, what can be garnered from existing genetic studies (although limited) suggests that genomic heterogeneity of the eCB-related genes may influence in part substance abuse vulnerability and relate to behavioural and pathophysiological traits that are highly associated with addictive disorders in humans (FIG. 5).

***CNR1*.** Human *CNR1* is located on 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, although each of the main mRNA variants expresses the same exon 4 that encodes the CB1R protein¹⁴⁸. Indeed, *CNR1* exhibits substantial functional conservation, with few common missense variants in the CB1R protein being expressed¹⁴⁸.

One of the first *CNR1* variants explored in relation to drug abuse was the AAT-triplet repeat ((AAT)_n) microsatellite polymorphism in the 3'-untranslated region, located close to the exon 4 translational start site¹⁴⁸. Unfortunately, direct functional evidence is lacking to understand its relevance to eCB processing, but the increased number of repeats is speculated to result in reduced CB1R expression¹⁴⁹. Increased frequency of long (AAT)_n was initially observed in an intravenous drug-dependent non-Hispanic US white population¹⁵⁰, and this was partially supported in subsequent evaluations of Afro-Caribbean individuals¹⁵¹. Some reports

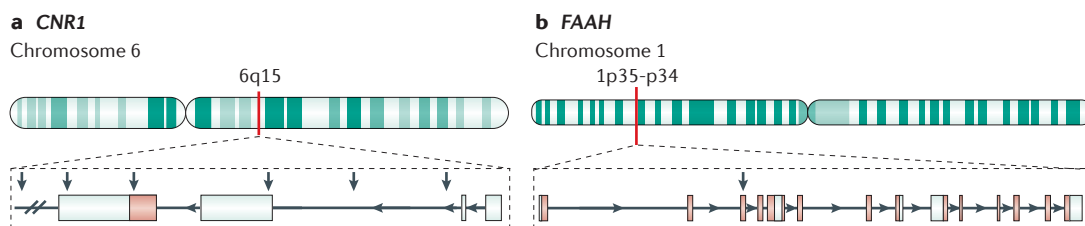


Figure 5 | *CNR1* and *FAAH* genes and genetic variants associated with addiction. The endocannabinoid (eCB) genes primarily studied to date in relation to genetic associations with addiction are *CNR1* (part **a**) and fatty acid amide hydrolase (*FAAH*) (part **b**). Human *CNR1*, which encodes cannabinoid 1 receptor (CB1R), 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 being located at the 5'-end of exon 4 (REFS 146–148). There are multiple mRNA variants of *CNR1*, with the prominent form encoding the canonical 472-amino-acid-long protein. The *FAAH* gene is located on human chromosome 1, 1p35-p34, and is transcribed from the plus strand (5'-to-3' orientation). The gene contains 15 exons, with functional protein domains being encoded across multiple exons. Recently, another *FAAH* gene, *FAAH2*, was identified on chromosome X in cytogenetic band Xp11.21; the encoded protein is composed of 532 amino acids and shares about 20% sequence identity with the canonical protein *FAAH1* (REF 147). There is evidence, although not all consistent, that genetic variants (arrows) associated with addiction and related phenotypes, such as reward sensitivity, impulsivity and negative affect, are located within exon 4 (rs1049353), introns (rs2023239, rs1535255 and rs806380) and the 3'-untranslated region (AAT-triplet repeat; rs806368) of *CNR1* (REFS 159, 146–148). For the *FAAH* gene, the polymorphic variant most associated with substance-use disorders is rs324420 (exon 3)^{166,146,147}.

failed to replicate the original finding^{148,152}, but a meta-analysis of multiple variants of *CNR1* in white populations specifically identified the (AAT)_n polymorphism ($n > 16$ repeats) as the only significant association with illicit SUDs¹⁵³. 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 abusers¹⁴⁶.

Additional single nucleotide polymorphisms (SNPs) of *CNR1* 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 in a way that could alter CB1R function. Several investigations, although 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 white patients¹⁵⁴ and enhanced impulsivity in Native Americans with a high lifetime prevalence of substance dependence¹⁵⁵. The G1359A variant has also been associated with heroin abuse in a white population, but with the A-allele conferring protection and the G/G genotype conferring addiction risk¹⁵⁶. Additional studies are clearly needed to determine whether the risk-versus-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 variation in the genome falls outside protein-coding regions¹⁵⁷. The rs2023239 variant, representing a T to C polymorphism in the intronic region upstream of exon 3, has been shown to relate to CB1R levels measured in post-mortem brain tissue¹⁵⁸ and *in vivo* using PET imaging⁹⁴, with the C-allele being associated with enhanced CB1R 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 abuse¹⁴⁸. Individuals with the C-allele use greater amounts of cannabis, exhibit higher cannabis dependency and experience greater negative affect and craving for cannabis following withdrawal¹⁵⁹. The rs2023239 minor allele also associates with increased activation in reward-associated brain areas (as measured by blood oxygenation level-dependent (BOLD) imaging) to cannabis-related cues¹⁶⁰. 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, and a strong association with alcohol-use disorder and craving measures¹⁶¹.

Several *CNR1* haplotype blocks have also been linked with addiction. When analysed as a haplotype (TAG), three SNPs (rs806379, rs1535255 and rs2023239) in the distal region of intron 2 of the *CNR1* gene were significantly associated with polysubstance abuse in adults from different ethnicities¹⁴⁸. Moreover, Agrawal *et al.*¹⁶² demonstrated an association between a *CNR1* haplotype (rs806380, rs806368 and rs754387) and cannabis dependence (the majority of these individuals also met criteria for alcohol dependence). The rs806380 SNP proximal to the TAG 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 and rs1049353: GGCC) or increased (TACC and GACC) risk for cannabis dependence¹⁶³. However, inconsistent and nominal significance has been reported in replication studies of cannabis dependence in adolescent and young adult populations¹⁶⁴ and for other haplotypes in substance abuse populations¹⁶⁵. Overall, although the majority of genetic investigations suggest

Cytogenetic band

A distinct region on the chromosome (visible microscopically after special staining).

Endophenotype

A term used to separate behavioural symptoms into stable phenotypes with a clear genetic basis, typically applicable to heritable disorders.

Haplotype blocks

Sets of DNA variations (or polymorphisms) that tend to be inherited together.

Post-translational histone modification

A covalent modification of histones that package and order DNA into nucleosomes. These modifications occur during or after histone biosynthesis.

an association between *CNR1* variants and aspects of SUDs, the data are not definitive and no causative loci have been described to date. What seems most consistent in the human genetic studies is a relevance to drug cue sensitivity and craving, which would complement the preclinical animal studies that demonstrate their direct link to the ECS.

FAAH. 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. Human *FAAH* is located on chromosome 1p35-p34 and has 15 exons, with functional protein domains being encoded across multiple exons. A SNP that has been highly investigated is rs324420, which is located in exon 3 and results in a missense mutation of a C–A replacement at position 385, leading to a proline to threonine change at protein position 129 (REF. 166). This C385A SNP is functional and is thought 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* substrates¹⁶⁷. Although some studies have not observed associations between the C385A polymorphism and SUDs, existing evidence implicates this genetic disruption in addiction-related behaviours in different races and ethnicities¹⁴⁷. Specifically, the A/A genotype associates with reduced vulnerability for cannabis dependence in white adults, whereas the C/C genotype associates with increased craving and negative affect during cannabis withdrawal. Initial studies failed to detect a link between

the A/A genotype and alcohol or nicotine dependence¹⁶⁸, although recently an over-representation of C/C carriers was observed among individuals consuming levels of alcohol that put them at increased risk of alcohol-related problems¹⁶⁹. Carriers of the *FAAH* C385A SNP display increased ventral striatal reactivity associated with delay discounting, a behavioural index of impulsivity and reward sensitivity¹⁷⁰ and a markedly decreased relationship between threat-related amygdala reactivity and trait anxiety, similar to patterns observed in individuals with high familial risk for alcoholism¹⁷¹. 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 sensitivity, impulsivity and negative affect, consistent with preclinical evidence linking the ECS to such behaviours. Gene–gene interactions within the ECS may also be relevant to vulnerability, as there seem 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 cannabis cues and more-severe negative affect during cannabis abstinence^{159,160}. Growing evidence of an eCB influence on epigenetic mechanisms suggests an additional but understudied way in which EC signalling may contribute to addiction (BOX 2). Clearly, a major confounding factor of most investigations to date is the small population size used, emphasizing the need for replication studies and studies using larger populations. The few existing agnostic genome-wide approaches have not identified eCB-related genes in relation to SUDs 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 behavioural traits limits the possibility to understand the full repertoire of the ECS to individual vulnerability to addiction. Moreover, the functional consequences of the variants (causal or correlated) are unknown, which makes coming to definitive conclusions challenging.

Summary and future directions

Although enhancement of eCB levels does not produce rewarding effects per se, eCB signalling at cannabinoid receptors participates in the mediation and modulation of both natural and drug-induced reward. Brain eCB content is modulated by most drugs of abuse and natural rewards, and a robust CB1R influence on the motivation to consume distinct classes of abused drugs and the association of *CNR1* polymorphisms with aberrant reward processing and addictive behaviours strongly implicate CB1Rs in the aetiology of addiction. Long-term drug use leads to neuroadaptive downregulation of eCB signalling resulting from diminished CB1R and/or CB2R function as well as possible disruptions in eCB biosynthesis and/or clearance. This blunting of eCB function may contribute to known susceptibility factors for relapse, namely, increased stress

Box 2 | Endocannabinoid influence on epigenetic mechanisms

Epigenetic influences are functionally relevant changes to the genome that do not involve disruptions in the nucleotide sequence of DNA. Examples of epigenetic mechanisms include DNA methylation, post-translational histone modification, nucleosome positioning and silencing associated with small non-coding RNAs (such as microRNAs and small interfering RNAs). Recent evidence demonstrates that epigenetic factors can regulate the expression of endocannabinoid (eCB)-related genes and that eCBs may themselves induce epigenetic alterations¹⁹⁴. For example, DNA hypermethylation of *CNR1* (the gene encoding cannabinoid 1 receptor (CB1R)) results in downregulation of transcription in the CNS and immune system, whereas decreased DNA methylation can result in increased fatty acid amide hydrolase (*FAAH*) transcription; these processes have been implicated in several pathologies, including colon cancer and late-onset Alzheimer disease. Conversely, eCB-induced alterations in enzymes influencing histone modification may disrupt the 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* promoter¹⁹⁵, which, through a resultant decrease in CB1R expression, could contribute to affective dysregulation and addiction susceptibility later in life. Several studies implicate increased epigenetic influences following chronic Δ^9 -tetrahydrocannabinol (Δ^9 -THC) exposure. Cannabis-dependent patients present robust methylation of the *CNR1* promoter in association with diminished *CNR1* mRNA in peripheral blood cells¹⁹⁶. Furthermore, offspring of maternal cannabis users exhibit histone methylation and dysregulated mesolimbic dopamine D₂ receptor expression¹⁹⁷, and adolescent Δ^9 -THC exposure is associated with nucleus accumbens (NAc) chromatin modifications and concurrent upregulation of the opioid neuropeptide proenkephalin gene¹⁹⁸. Prenatal alcohol exposure increases expression of the regulatory microRNA miR-26b (which targets the 3'-untranslated region of the *CNR1* transcript) and decreased *CNR1* transcription in the adult mouse brain¹⁹⁹. Thus, growing evidence suggests that there are eCB-related epigenetic influences following drug exposure.

responsivity, increased negative affect, inefficient extinction of drug-related memories and increased drug-seeking behaviour and drug craving. Recent preclinical evidence demonstrates the efficacy of eCB-clearance inhibitors for ameliorating these behavioural abnormalities, which might offer future therapeutic interventions for addiction disorders. Importantly, because eCBs are generally produced in a synapse-specific manner, eCB-clearance inhibitors may preferentially facilitate eCB signalling in specific circuits engaged by distinct stimuli (for example, stress- or drug-associated cues) and therefore could present fewer unwanted behavioural effects than are produced by exogenous agonists that produce widespread cannabinoid receptor activation.

Despite growing attention being given to the cannabinoids, there are still notable gaps in our understanding of the eCB influence on reward and addiction. The ECS plays a prominent part in neuronal guidance and brain development¹⁷² and, as such, disruptions in eCB function at an early age probably have 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}. Although the effects of early life exposure to non-cannabinoid drugs are well studied, the specific contributions of persistent drug-induced disruptions in eCB signalling on adult neural function and behaviour are not understood. Robust bidirectional interactions between the ECS and sex hormones are now recognized¹⁷⁵, but few studies have characterized possible sex differences in the eCB influence on reward function, addiction and cognitive processing. There are also substantial limitations in the interpretation and replication of genetic analyses of the eCB influence in addiction, owing to heterogeneity of the populations, drug classes, polysubstance use and even drug-use phenotypes examined. Large-scale future studies across different populations and drug classes will be critical to understanding the relative effect and causal nature of ECS-related genetic mutations in the vulnerability to addictive disorders. Filling these gaps of knowledge is critical, given the important need for scientific data to help guide current discussions and changes being made in marijuana-legalization policies.

- Glass, M., Dragunow, M. & Faull, R. L. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* **77**, 299–318 (1997).
 - Wang, X., Dow-Edwards, D., Keller, E. & Hurd, Y. L. Preferential limbic expression of the cannabinoid receptor mRNA in the human fetal brain. *Neuroscience* **118**, 681–694 (2003).
- References 1 and 2 demonstrate in human fetal and adult brains the anatomical expression of cannabinoid receptors, which are important targets for eCB and exogenous cannabinoid ligands.**
- Atwood, B. K. & Mackie, K. CB₂: a cannabinoid receptor with an identity crisis. *Br. J. Pharmacol.* **160**, 467–479 (2010).
 - Salamone, J. D., Correa, M., Mingote, S. M. & Weber, S. M. Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. *Curr. Opin. Pharmacol.* **5**, 34–41 (2005).
 - Koob, G. F. & Volkow, N. D. Neurocircuitry of addiction. *Neuropsychopharmacology* **35**, 217–238 (2010).
 - Everitt, B. J. *et al.* Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philos. Trans. R. Soc. B* **363**, 3125–3135 (2008).
 - Carlezon, W. A. Jr & Thomas, M. J. Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis. *Neuropharmacology* **56** (Suppl. 1), 122–132 (2009).
 - Iemolo, A. *et al.* CRF–CRF₁ receptor system in the central and basolateral nuclei of the amygdala differentially mediates excessive eating of palatable food. *Neuropsychopharmacology* **38**, 2456–2466 (2013).
 - Herkenham, M. *et al.* Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J. Neurosci.* **11**, 563–583 (1991).
- One of the first studies to visualize cannabinoid receptors in the rodent brain and show that they are some of the most-abundant G protein-coupled receptors in the brain.**
- Sidhpura, N. & Parsons, L. H. Endocannabinoid-mediated synaptic plasticity and addiction-related behavior. *Neuropharmacology* **61**, 1070–1087 (2011).
 - Panagis, G., Mackey, B. & Vlachou, S. Cannabinoid regulation of brain reward processing with an emphasis on the role of CB₁ receptors: a step back into the future. *Front. Psychiatry* **5**, 92 (2014).
 - Solinas, M., Justinova, Z., Goldberg, S. R. & Tanda, G. Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. *J. Neurochem.* **98**, 408–419 (2006).
 - Melis, M. & Pistis, M. Hub and switches: endocannabinoid signalling in midbrain dopamine neurons. *Philos. Trans. R. Soc. B* **367**, 3276–3285 (2012).
 - Silvestri, C. & Di Marzo, V. The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. *Cell. Metab.* **17**, 475–490 (2013).
 - Klein, C., Hill, M. N., Chang, S. C., Hillard, C. J. & Gorzalka, B. B. Circulating endocannabinoid concentrations and sexual arousal in women. *J. Sex. Med.* **9**, 1588–1601 (2012).
 - Trezza, V., Baarendse, P. J. & Vanderschuren, L. J. The pleasures of play: pharmacological insights into social reward mechanisms. *Trends Pharmacol. Sci.* **31**, 463–469 (2010).
 - Fattore, L. *et al.* Cannabinoids and reward: interactions with the opioid system. *Crit. Rev. Neurobiol.* **16**, 147–158 (2004).
 - Di Marzo, V. *et al.* Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* **410**, 822–825 (2001).
 - Mechoulam, R., Hanus, L. O., Pertwee, R. & Howlett, A. C. Early phytocannabinoid chemistry to endocannabinoids and beyond. *Nat. Rev. Neurosci.* **15**, 757–764 (2014).
 - Vlachou, S. & Panagis, G. Regulation of brain reward by the endocannabinoid system: a critical review of behavioral studies in animals. *Curr. Pharm. Des.* **20**, 2072–2088 (2014).
 - Panlilio, L. V., Justinova, Z. & Goldberg, S. R. Inhibition of FAAH and activation of PPAR: new approaches to the treatment of cognitive dysfunction and drug addiction. *Pharmacol. Ther.* **138**, 84–102 (2013).
 - Wiley, J. L. *et al.* Endocannabinoid contribution to Δ^9 -tetrahydrocannabinol discrimination in rodents. *Eur. J. Pharmacol.* **737**, 97–105 (2014).
 - Long, J. Z. *et al.* Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk *in vivo*. *Proc. Natl Acad. Sci. USA* **106**, 20270–20275 (2009).
 - Justinova, Z. *et al.* Effects of fatty acid amide hydrolase (FAAH) inhibitors in non-human primate models of nicotine reward and relapse. *Neuropsychopharmacology* **40**, 2185–2197 (2015).
 - Serrano, A. & Parsons, L. H. Endocannabinoid influence in drug reinforcement, dependence and addiction-related behaviors. *Pharmacol. Ther.* **132**, 215–241 (2011).
 - Ellgren, M., Spano, S. M. & Hurd, Y. L. Adolescent cannabis exposure alters opiate intake and opioid limbic neuronal populations in adult rats. *Neuropsychopharmacology* **32**, 607–615 (2007).
- This study was among the first to demonstrate that adolescent Δ^9 -THC exposure results in enhanced μ -opioid receptor signalling in the NAc, which contributes to increased heroin self-administration.**
- Cheer, J. F. *et al.* Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. *J. Neurosci.* **27**, 791–795 (2007).
- One of the first studies to demonstrate a common CB1R–DA interaction contributing to the dopaminergic effects produced by abused substances with distinct pharmacological properties.**
- Alvarez-Jaimes, L. & Parsons, L. H. Regional influence of CB₁ receptor signaling on ethanol self-administration by rats. *Open Neuropsychopharmacol. J.* **2**, 77–85 (2009).
 - Simonnet, A., Cador, M. & Caille, S. Nicotine reinforcement is reduced by cannabinoid CB1 receptor blockade in the ventral tegmental area. *Addict. Biol.* **18**, 930–936 (2013).
 - Caille, S., Alvarez-Jaimes, L., Polis, I., Stouffer, D. G. & Parsons, L. H. Specific alterations of extracellular endocannabinoid levels in the nucleus accumbens by ethanol, heroin, and cocaine self-administration. *J. Neurosci.* **27**, 3695–3702 (2007).
- Provides the first *in vivo* evidence that voluntary self-administration of non-cannabinoid drugs produces drug-specific and dose-reliant alterations in extracellular AEA and 2-AG levels in the rat NAc.**
- D'Souza, M. S. & Markou, A. Neuronal mechanisms underlying development of nicotine dependence: implications for novel smoking-cessation treatments. *Addict. Sci. Clin. Pract.* **6**, 4–16 (2011).
 - Koob, G. F. Theoretical frameworks and mechanistic aspects of alcohol addiction: alcohol addiction as a reward deficit disorder. *Curr. Top. Behav. Neurosci.* **13**, 3–30 (2013).
 - Shippenberg, T. S. & Elmer, G. I. The neurobiology of opiate reinforcement. *Crit. Rev. Neurobiol.* **12**, 267–303 (1998).
 - Caille, S. & Parsons, L. H. Cannabinoid modulation of opiate reinforcement through the ventral striatopallidum pathway. *Neuropsychopharmacology* **31**, 804–813 (2006).
 - Vlachou, S., Nomikos, G. G. & Panagis, G. WIN 55,212-2 decreases the reinforcing actions of cocaine through CB₁ cannabinoid receptor stimulation. *Behav. Brain Res.* **141**, 215–222 (2003).
 - Fattore, L., Martellotta, M. C., Cossu, G., Mascia, M. S. & Fratta, W. CB1 cannabinoid receptor agonist WIN 55,212-2 decreases intravenous cocaine self-administration in rats. *Behav. Brain Res.* **104**, 141–146 (1999).

37. Olieri, S., Joliet-Riopel, A., Potvin, S. & Jutras-Aswad, D. Modulation of the endocannabinoid system: vulnerability factor and new treatment target for stimulant addiction. *Front. Psychiatry* **4**, 109 (2013).
38. Xi, Z. X. *et al.* Brain cannabinoid CB₂ receptors modulate cocaine's actions in mice. *Nat. Neurosci.* **14**, 1160–1166 (2011).
Provides the first evidence that drug reward can be modulated by changes in brain CB₂R activity.
39. Zhang, H. Y. *et al.* Species differences in cannabinoid receptor 2 and receptor responses to cocaine self-administration in mice and rats. *Neuropsychopharmacology* **40**, 1037–1051 (2014).
Characterizes important species differences in the splicing and expression of CB₂R genes and receptor structures relevant to the different effects noted for CB₂R-selective ligands in mice and rats.
40. Ignatowska-Jankowska, B. M., Muldoon, P. P., Lichtman, A. H. & Damaj, M. I. The cannabinoid CB₂ receptor is necessary for nicotine-conditioned place preference, but not other behavioral effects of nicotine in mice. *Psychopharmacology* **229**, 591–601 (2013).
41. Navarrete, F. *et al.* Role of CB₂ cannabinoid receptors in the rewarding, reinforcing, and physical effects of nicotine. *Neuropsychopharmacology* **38**, 2515–2524 (2013).
42. Adamczyk, P. *et al.* The effects of cannabinoid CB₁, CB₂ and vanilloid TRPV1 receptor antagonists on cocaine addictive behavior in rats. *Brain Res.* **1444**, 45–54 (2012).
43. Gamaledin, I., Zvonok, A., Makriyannis, A., Goldberg, S. R. & Le Foll, B. Effects of a selective cannabinoid CB₂ agonist and antagonist on intravenous nicotine self administration and reinstatement of nicotine seeking. *PLoS ONE* **7**, e29900 (2012).
44. Pava, M. J. & Woodward, J. J. A review of the interactions between alcohol and the endocannabinoid system: implications for alcohol dependence and future directions for research. *Alcohol* **46**, 185–204 (2012).
45. Ceccarini, J., Casteels, C., Koole, M., Bormans, G. & Van Laere, K. Transient changes in the endocannabinoid system after acute and chronic ethanol exposure and abstinence in the rat: a combined PET and microdialysis study. *Eur. J. Nucl. Med. Mol. Imaging* **40**, 1582–1594 (2013).
46. Alvarez-Jaimes, L., Stouffer, D. G. & Parsons, L. H. Chronic ethanol treatment potentiates ethanol-induced increases in interstitial nucleus accumbens endocannabinoid levels in rats. *J. Neurochem.* **111**, 37–48 (2009).
47. Malinen, H. & Hyttia, P. Ethanol self-administration is regulated by CB₁ receptors in the nucleus accumbens and ventral tegmental area in alcohol-preferring AA rats. *Alcohol Clin. Exp. Res.* **32**, 1976–1983 (2008).
48. Gonzalez, S. *et al.* Changes in endocannabinoid contents in the brain of rats chronically exposed to nicotine, ethanol or cocaine. *Brain Res.* **954**, 73–81 (2002).
49. Buczynski, M. W., Polis, I. Y. & Parsons, L. H. The volitional nature of nicotine exposure alters anandamide and oleoylethanolamide levels in the ventral tegmental area. *Neuropsychopharmacology* **38**, 574–584 (2013).
50. Viganò, D. *et al.* Chronic morphine modulates the contents of the endocannabinoid, 2-arachidonoyl glycerol, in rat brain. *Neuropsychopharmacology* **28**, 1160–1167 (2003).
51. Viganò, D. *et al.* Changes in endocannabinoid levels in a rat model of behavioural sensitization to morphine. *Eur. J. Neurosci.* **20**, 1849–1857 (2004).
52. Patel, S., Rademacher, D. J. & Hillard, C. J. Differential regulation of the endocannabinoids anandamide and 2-arachidonoyl glycerol within the limbic forebrain by dopamine receptor activity. *J. Pharmacol. Exp. Ther.* **306**, 880–888 (2003).
53. Bystrowska, B., Smaga, I., Frankowska, M. & Filip, M. Changes in endocannabinoid and N-acyl ethanolamine levels in rat brain structures following cocaine self-administration and extinction training. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **50**, 1–10 (2014).
54. Rivera, P. *et al.* Cocaine self-administration differentially modulates the expression of endogenous cannabinoid system-related proteins in the hippocampus of Lewis versus Fischer 344 rats. *Int. J. Neuropsychopharmacol.* **16**, 1277–1293 (2013).
55. Palomino, A. *et al.* Effects of acute versus repeated cocaine exposure on the expression of endocannabinoid signaling-related proteins in the mouse cerebellum. *Front. Integr. Neurosci.* **8**, 22 (2014).
56. Gulyas, A. I. *et al.* Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur. J. Neurosci.* **20**, 441–458 (2004).
One of the first studies to characterize the cellular location of the eCB metabolic enzymes, thereby emphasizing potentially distinct influences of AEA and 2-AG processing on synaptic function.
57. Kim, J. & Alger, B. E. Reduction in endocannabinoid tone is a homeostatic mechanism for specific inhibitory synapses. *Nat. Neurosci.* **13**, 592–600 (2010).
58. Merritt, L. L., Martin, B. R., Walters, C., Lichtman, A. H. & Damaj, M. I. The endogenous cannabinoid system modulates nicotine reward and dependence. *J. Pharmacol. Exp. Ther.* **326**, 483–492 (2008).
59. Muldoon, P. P., Lichtman, A. H., Parsons, L. H. & Damaj, M. I. The role of fatty acid amide hydrolase inhibition in nicotine reward and dependence. *Life Sci.* **92**, 458–462 (2013).
60. Scherma, M. *et al.* Inhibition of anandamide hydrolysis by cyclohexyl carbamic acid 3'-carbamoyl-3-yl ester (URB597) reverses abuse-related behavioral and neurochemical effects of intoxication in rats. *J. Pharmacol. Exp. Ther.* **327**, 482–490 (2008).
Provides the first evidence that FAAH inhibition reduces nicotine reward in rats. This finding led to a series of studies demonstrating that stimulation of PPARα receptors by FAAH substrates, including oleoylethanolamide, prevents nicotine-induced activation of the mesolimbic DA system in rats and non-human primates (these collective studies are reviewed and discussed in reference 61).
61. Melis, M. & Pistis, M. Targeting the interaction between fatty acid ethanolamides and nicotinic receptors: therapeutic perspectives. *Pharmacol. Res.* **86**, 42–49 (2014).
62. Blednov, Y. A., Cravatt, B. F., Boehm, S. L. 2nd, Walker, D. & Harris, R. A. Role of endocannabinoids in alcohol consumption and intoxication: studies of mice lacking fatty acid amide hydrolase. *Neuropsychopharmacology* **32**, 1570–1582 (2007).
63. Vinod, K. Y., Sanguino, E., Yalamanchili, R., Manzanares, J. & Hungund, B. L. Manipulation of fatty acid amide hydrolase functional activity alters sensitivity and dependence to ethanol. *J. Neurochem.* **104**, 233–243 (2008).
64. Hansson, A. C. *et al.* Genetic impairment of frontocortical endocannabinoid degradation and high alcohol preference. *Neuropsychopharmacology* **32**, 117–126 (2007).
65. Cippitelli, A. *et al.* Increase of brain endocannabinoid anandamide levels by FAAH inhibition and alcohol abuse behaviours in the rat. *Psychopharmacology* **198**, 449–460 (2008).
66. Justinova, Z. *et al.* Fatty acid amide hydrolase inhibition heightens anandamide signaling without producing reinforcing effects in primates. *Biol. Psychiatry* **64**, 930–937 (2008).
67. Luchicchi, A. *et al.* Effects of fatty acid amide hydrolase inhibition on neuronal responses to nicotine, cocaine and morphine in the nucleus accumbens shell and ventral tegmental area: involvement of PPARα nuclear receptors. *Addict. Biol.* **15**, 277–288 (2010).
68. Lovinger, D. M. Presynaptic modulation by endocannabinoids. *Handb. Exp. Pharmacol.* **184**, 435–477 (2008).
69. Mereu, M. *et al.* Cocaine-induced endocannabinoid release modulates behavioral and neurochemical sensitization in mice. *Addict. Biol.* **20**, 91–103 (2013).
70. Scherma, M. *et al.* The anandamide transport inhibitor AM404 reduces the rewarding effects of nicotine and nicotine-induced dopamine elevations in the nucleus accumbens shell in rats. *Br. J. Pharmacol.* **165**, 2539–2548 (2012).
71. Gamaledin, I., Guranda, M., Goldberg, S. R. & Le Foll, B. The selective anandamide transport inhibitor VDM11 attenuates reinstatement of nicotine seeking behaviour, but does not affect nicotine intake. *Br. J. Pharmacol.* **164**, 1652–1660 (2011).
72. Gamaledin, I. *et al.* AM404 attenuates reinstatement of nicotine seeking induced by nicotine-associated cues and nicotine priming but does not affect nicotine- and food-taking. *J. Psychopharmacol.* **27**, 564–571 (2013).
73. Vlachou, S., Stamatopoulou, F., Nomikos, G. G. & Panagis, G. Enhancement of endocannabinoid neurotransmission through CB₁ cannabinoid receptors counteracts the reinforcing and psychostimulant effects of cocaine. *Int. J. Neuropsychopharmacol.* **11**, 905–923 (2008).
74. Ducci, F. & Goldman, D. The genetic basis of addictive disorders. *Psychiatr. Clin. North Am.* **35**, 495–519 (2012).
75. Nestler, E. J. Epigenetic mechanisms of drug addiction. *Neuropharmacology* **76**, 259–268 (2014).
76. Hirvonen, J. *et al.* Reversible and regionally selective downregulation of brain cannabinoid CB₁ receptors in chronic daily cannabis smokers. *Mol. Psychiatry* **17**, 642–649 (2012).
Provides the first *in vivo* evidence of regionally selective down-regulation of brain CB₁R in human cannabis smokers.
77. Ceccarini, J. *et al.* [¹⁸F]MK-9470 PET measurement of cannabinoid CB₁ receptor availability in chronic cannabis users. *Addict. Biol.* **20**, 357–367 (2015).
78. Breivogel, C. S. *et al.* Chronic Δ⁹-tetrahydrocannabinol treatment produces a time-dependent loss of cannabinoid receptors and cannabinoid receptor-activated G proteins in rat brain. *J. Neurochem.* **73**, 2447–2459 (1999).
79. Sim, L. J., Hampson, R. E., Deadwyler, S. A. & Childers, S. R. Effects of chronic treatment with Δ⁹-tetrahydrocannabinol on cannabinoid-stimulated [³⁵S]GTPγS autoradiography in rat brain. *J. Neurosci.* **16**, 8057–8066 (1996).
80. Dudok, B. *et al.* Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling. *Nat. Neurosci.* **18**, 75–86 (2015).
Uses nanoscale imaging and electrophysiological techniques to demonstrate that there is greater CB₁R expression and influence on perisomatically projecting versus dendritically projecting GABA interneurons in the mouse hippocampus and that persistent deficits in hippocampal LTP following chronic Δ⁹-THC exposure result from near-complete loss of CB₁R at somatic synapses.
81. Puighermanal, E., Busquets-Garcia, A., Maldonado, R. & Ozaita, A. Cellular and intracellular mechanisms involved in the cognitive impairment of cannabinoids. *Phil. Trans. R. Soc. B* **367**, 3254–3263 (2012).
82. Di Marzo, V. *et al.* Enhancement of anandamide formation in the limbic forebrain and reduction of endocannabinoid contents in the striatum of Δ⁹-tetrahydrocannabinol-tolerant rats. *J. Neurochem.* **74**, 1627–1635 (2000).
83. Schlosburg, J. E. *et al.* Inhibitors of endocannabinoid-metabolizing enzymes reduce precipitated withdrawal responses in THC-dependent mice. *AAPS J.* **11**, 342–352 (2009).
84. Leweke, F. M. *et al.* Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. *Schizophr. Res.* **94**, 29–36 (2007).
85. Morgan, C. J. *et al.* Cerebrospinal fluid anandamide levels, cannabis use and psychotic-like symptoms. *Br. J. Psychiatry* **202**, 381–382 (2013).
86. Muhl, D. *et al.* Increased CB₁ mRNA and anandamide in human blood after cessation of cannabis abuse. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **387**, 691–695 (2014).
87. Lutz, B. Endocannabinoid signals in the control of emotion. *Curr. Opin. Pharmacol.* **9**, 46–52 (2009).
88. Murillo-Rodriguez, E. *et al.* The emerging role of the endocannabinoid system in the sleep-wake cycle modulation. *Cent. Nerv. Syst. Agents Med. Chem.* **11**, 189–196 (2011).
89. Budney, A. J., Hughes, J. R., Moore, B. A. & Novy, P. L. Marijuana abstinence effects in marijuana smokers maintained in their home environment. *Arch. Gen. Psychiatry* **58**, 917–924 (2001).
90. Gates, P., Albertella, L. & Copeland, J. Cannabis withdrawal and sleep: a systematic review of human studies. *Subst. Abuse*. <http://dx.doi.org/10.1080/08897077.2015.1023484> (2015).
91. Serrano, A. *et al.* Differential effects of single versus repeated alcohol withdrawal on the expression of endocannabinoid system-related genes in the rat amygdala. *Alcohol Clin. Exp. Res.* **36**, 984–994 (2012).
92. Mitrirattanakul, S. *et al.* Bidirectional alterations of hippocampal cannabinoid 1 receptors and their endogenous ligands in a rat model of alcohol withdrawal and dependence. *Alcohol Clin. Exp. Res.* **31**, 855–867 (2007).
93. Vinod, K. Y. *et al.* Selective alterations of the CB₁ receptors and the fatty acid amide hydrolase in the ventral striatum of alcoholics and suicides. *J. Psychiatr. Res.* **44**, 591–597 (2010).
94. Hirvonen, J. *et al.* Reduced cannabinoid CB₁ receptor binding in alcohol dependence measured with positron emission tomography. *Mol. Psychiatry* **18**, 916–921 (2013).

95. Ceccarini, J. *et al.* Changes in cerebral CB₁ receptor availability after acute and chronic alcohol abuse and monitored abstinence. *J. Neurosci.* **34**, 2822–2831 (2014).
96. Neumeister, A. *et al.* Positron emission tomography shows elevated cannabinoid CB₁ receptor binding in men with alcohol dependence. *Alcohol Clin. Exp. Res.* **36**, 2104–2109 (2012).
This study was among the first to demonstrate altered brain CB1R availability in humans with alcoholism.
97. Vinod, K. Y., Yalamanchili, R., Xie, S., Cooper, T. B. & Hungund, B. L. Effect of chronic ethanol exposure and its withdrawal on the endocannabinoid system. *Neurochem. Int.* **49**, 619–625 (2006).
98. Werling, L. L., Reed, S. C., Wade, D. & Izenwasser, S. Chronic nicotine alters cannabinoid-mediated locomotor activity and receptor density in periadolescent but not adult male rats. *Int. J. Dev. Neurosci.* **27**, 263–269 (2009).
99. Marco, E. M. *et al.* Subchronic nicotine exposure in adolescence induces long-term effects on hippocampal and striatal cannabinoid-CB₁ and mu-opioid receptors in rats. *Eur. J. Pharmacol.* **557**, 37–43 (2007).
100. Rubino, T., Tizzoni, L., Viganò, D., Massi, P. & Parolaro, D. Modulation of rat brain cannabinoid receptors after chronic morphine treatment. *Neuroreport* **8**, 3219–3223 (1997).
101. Cichewicz, D. L., Haller, V. L. & Welch, S. P. Changes in opioid and cannabinoid receptor protein following short-term combination treatment with Δ^9 -tetrahydrocannabinol and morphine. *J. Pharmacol. Exp. Ther.* **297**, 121–127 (2001).
102. Adamczyk, P. *et al.* Long-lasting increase in [³H] CP55,940 binding to CB1 receptors following cocaine self-administration and its withdrawal in rats. *Brain Res.* **1451**, 34–43 (2012).
103. Pavon, F. J. *et al.* Evaluation of plasma-free endocannabinoids and their congeners in abstinent cocaine addicts seeking outpatient treatment: impact of psychiatric co-morbidity. *Addict. Biol.* **18**, 955–969 (2013).
104. Heifets, B. D. & Castillo, P. E. Endocannabinoid signaling and long-term synaptic plasticity. *Annu. Rev. Physiol.* **71**, 283–306 (2009).
105. Adermark, L., Jonsson, S., Ericson, M. & Soderpalm, B. Intermittent ethanol consumption depresses endocannabinoid-signaling in the dorsolateral striatum of rat. *Neuropharmacology* **61**, 1160–1165 (2011).
106. Clarke, R. B. & Adermark, L. Acute ethanol treatment prevents endocannabinoid-mediated long-lasting disinhibition of striatal output. *Neuropharmacology* **58**, 799–805 (2010).
107. DePoy, L. *et al.* Chronic alcohol produces neuroadaptations to prime dorsal striatal learning. *Proc. Natl Acad. Sci. USA* **110**, 14783–14788 (2013).
108. Fourgeaud, L. *et al.* A single *in vivo* exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens. *J. Neurosci.* **24**, 6939–6945 (2004).
109. Pan, B., Hillard, C. J. & Liu, Q. S. Endocannabinoid signaling mediates cocaine-induced inhibitory synaptic plasticity in midbrain dopamine neurons. *J. Neurosci.* **28**, 1385–1397 (2008).
110. Liu, Q. S., Pu, L. & Poo, M. M. Repeated cocaine exposure *in vivo* facilitates LTP induction in midbrain dopamine neurons. *Nature* **437**, 1027–1031 (2005).
111. Grueter, B. A. *et al.* Extracellular-signal regulated kinase 1-dependent metabotropic glutamate receptor 5-induced long-term depression in the bed nucleus of the stria terminalis is disrupted by cocaine administration. *J. Neurosci.* **26**, 3210–3219 (2006).
112. McElligott, Z. A. & Winder, D. G. Modulation of glutamatergic synaptic transmission in the bed nucleus of the stria terminalis. *Prog. Neurobiopharmacol. Biol. Psychiatry* **33**, 1329–1335 (2009).
113. Hoffman, A. F., Oz, M., Caulder, T. & Lupica, C. R. Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. *J. Neurosci.* **23**, 4815–4820 (2003).
114. Mato, S. *et al.* A single *in-vivo* exposure to Δ^9 THC blocks endocannabinoid-mediated synaptic plasticity. *Nat. Neurosci.* **7**, 585–586 (2004).
References 113 and 114 were among the first to demonstrate that Δ^9 -THC exposure disrupts synaptic plasticity of NAc neurons in rodents.
115. Reisiger, A. R. *et al.* Nicotine self-administration induces CB1-dependent LTP in the bed nucleus of the stria terminalis. *J. Neurosci.* **34**, 4285–4292 (2014).
116. Koob, G. & Kreek, M. J. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *Am. J. Psychiatry* **164**, 1149–1159 (2007).
117. Morena, M., Patel, S., Bains, J. S. & Hill, M. N. Neurobiological interactions between stress and the endocannabinoid system. *Neuropsychopharmacology* <http://dx.doi.org/10.1038/npp.2015.166> (2015).
118. Ruehle, S., Rey, A. A., Remmers, F. & Lutz, B. The endocannabinoid system in anxiety, fear memory and habituation. *J. Psychopharmacol.* **26**, 23–39 (2012).
119. Bura, S. A., Burokas, A., Martín-García, E. & Maldonado, R. Effects of chronic nicotine on food intake and anxiety-like behaviour in CB₁ knockout mice. *Eur. Neuropsychopharmacol.* **20**, 369–378 (2010).
120. Cippitelli, A. *et al.* Endocannabinoid regulation of acute and protracted nicotine withdrawal: effect of FAAH inhibition. *PLoS ONE* **6**, e28142 (2011).
121. Mannucci, C. *et al.* Interactions between endocannabinoid and serotonergic systems in mood disorders caused by nicotine withdrawal. *Nicotine Tob. Res.* **13**, 239–247 (2011).
122. McKinzie, D. L. *et al.* Acoustic startle and fear-potentiated startle in alcohol-preferring (P) and non-preferring (NP) lines of rats. *Pharmacol. Biochem. Behav.* **65**, 691–696 (2000).
123. Barrenha, G. D. & Chester, J. A. Genetic correlation between innate alcohol preference and fear-potentiated startle in selected mouse lines. *Alcohol Clin. Exp. Res.* **31**, 1081–1088 (2007).
124. Powers, M. S., Barrenha, G. D., Milnac, N. S., Barker, E. L. & Chester, J. A. Effects of the novel endocannabinoid uptake inhibitor, LY2183240, on fear-potentiated startle and alcohol-seeking behaviors in mice selectively bred for high alcohol preference. *Psychopharmacology* **212**, 571–583 (2010).
125. Gunduz-Cinar, O., Hill, M. N., McEwen, B. S. & Holmes, A. Amygdala FAAH and anandamide: mediating protection and recovery from stress. *Trends Pharmacol. Sci.* **34**, 637–644 (2013).
126. Marsicano, G. & Lafenetre, P. Roles of the endocannabinoid system in learning and memory. *Curr. Top. Behav. Neurosci.* **1**, 201–230 (2009).
127. Carter, B. L. & Tiffany, S. T. Cue-reactivity and the future of addiction research. *Addiction* **94**, 349–351 (1999).
128. De Vries, T. J. & Schoffelmeer, A. N. Cannabinoid CB₁ receptors control conditioned drug seeking. *Trends Pharmacol. Sci.* **26**, 420–426 (2005).
This review details seminal studies by the authors and others demonstrating that CB1R blockade attenuates relapse-like behaviour in rats, thus paving the way for numerous studies demonstrating a potent influence of CB1R signalling on relapse-like behaviour induced both by drug exposure and by drug-paired conditioned cues across multiple classes of abused drugs.
129. Fattore, L. *et al.* An endocannabinoid mechanism in relapse to drug seeking: a review of animal studies and clinical perspectives. *Brain Res. Rev.* **53**, 1–16 (2007).
130. Alvarez-Jaimes, L., Polis, I. & Parsons, L. H. Attenuation of cue-induced heroin-seeking behavior by cannabinoid CB₁ antagonist infusions into the nucleus accumbens core and prefrontal cortex, but not basolateral amygdala. *Neuropsychopharmacology* **33**, 2483–2493 (2008).
131. Kostas, E., Cohen, C., Louis, C. & Griebel, G. Cortico-limbic circuitry for conditioned nicotine-seeking behavior in rats involves endocannabinoid signaling. *Psychopharmacology* **194**, 161–171 (2007).
132. De Vries, T. J., de Vries, W., Janssen, M. C. & Schoffelmeer, A. N. Suppression of conditioned nicotine and sucrose seeking by the cannabinoid-1 receptor antagonist SR141716A. *Behav. Brain Res.* **161**, 164–168 (2005).
133. Ward, S. J., Rosenberg, M., Dykstra, L. A. & Walker, E. A. The CB1 antagonist rimonabant (SR141716) blocks cue-induced reinstatement of cocaine seeking and other context and extinction phenomena predictive of relapse. *Drug Alcohol Depend.* **105**, 248–255 (2009).
134. Xi, Z. X. *et al.* Cannabinoid CB₁ receptor antagonist AM251 inhibits cocaine-primed relapse in rats: role of glutamate in the nucleus accumbens. *J. Neurosci.* **26**, 8531–8536 (2006).
135. De Petrocellis, L., Bisogno, T., Davis, J. B., Pertwee, R. G. & Di Marzo, V. Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide uptake with negligible capsaicin-like activity. *FEBS Lett.* **483**, 52–56 (2000).
136. van der Stelt, M. *et al.* Endocannabinoids and β -amyloid-induced neurotoxicity *in vivo*: effect of pharmacological elevation of endocannabinoid levels. *Cell. Mol. Life Sci.* **63**, 1410–1424 (2006).
137. Cippitelli, A. *et al.* The anandamide transport inhibitor AM404 reduces ethanol self-administration. *Eur. J. Neurosci.* **26**, 476–486 (2007).
138. Mangieri, R. A., Hong, K. I., Piomelli, D. & Sinha, R. An endocannabinoid signal associated with desire for alcohol is suppressed in recently abstinent alcoholics. *Psychopharmacology* **205**, 63–72 (2009).
139. Conklin, C. A. & Tiffany, S. T. Applying extinction research and theory to cue-exposure addiction treatments. *Addiction* **97**, 155–167 (2002).
140. Crombag, H. S. & Shaham, Y. Renewal of drug seeking by contextual cues after prolonged extinction in rats. *Behav. Neurosci.* **116**, 169–173 (2002).
141. Marsicano, G. *et al.* The endogenous cannabinoid system controls extinction of aversive memories. *Nature* **418**, 530–534 (2002).
Supplies the first evidence for a strong influence of CB1Rs in the amygdala in extinction learning related to fear, and this has led to critical insight into eCB mechanisms related to emotional pathologies, including anxiety disorders and post-traumatic stress disorder.
142. Lutz, B. The endocannabinoid system and extinction learning. *Mol. Neurobiol.* **36**, 92–101 (2007).
143. Kaplan, G. B., Heinrichs, S. C. & Carey, R. J. Treatment of addiction and anxiety using extinction approaches: neural mechanisms and their treatment implications. *Pharmacol. Biochem. Behav.* **97**, 619–625 (2011).
144. Kendler, K. S., Karkowski, L. M., Neale, M. C. & Prescott, C. A. Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. *Arch. Gen. Psychiatry* **57**, 261–269 (2000).
145. Agrawal, A. & Lynskey, M. T. Are there genetic influences on addiction: evidence from family, adoption and twin studies. *Addiction* **103**, 1069–1081 (2008).
146. Hillard, C. J., Weinlander, K. M. & Stuhr, K. L. Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence. *Neuroscience* **204**, 207–229 (2012).
147. Lopez-Moreno, J. A., Echeverry-Alzate, V. & Buhler, K. M. The genetic basis of the endocannabinoid system and drug addiction in humans. *J. Psychopharmacol.* **26**, 133–143 (2012).
148. Zhang, P. W. *et al.* Human cannabinoid receptor 1: 5' exons, candidate regulatory regions, polymorphisms, haplotypes and association with polysubstance abuse. *Mol. Psychiatry* **9**, 916–931 (2004).
Provides detailed structural characterization of human CNR1, thereby providing information on splice variants and novel SNPs as well as evidence of a haplotype-specific association between CNR1 mRNA expression and substance abuse phenotype.
149. Schroth, G. P., Chou, P. J. & Ho, P. S. Mapping Z-DNA in the human genome. Computer-aided mapping reveals a nonrandom distribution of potential Z-DNA-forming sequences in human genes. *J. Biol. Chem.* **267**, 11846–11855 (1992).
150. Comings, D. E. *et al.* Cannabinoid receptor gene (CNR1): association with i.v. drug use. *Mol. Psychiatry* **2**, 161–168 (1997).
151. Ballon, N. *et al.* (AAT)n repeat in the cannabinoid receptor gene (CNR1): association with cocaine addiction in an African-Caribbean population. *Pharmacogenomics* **6**, 126–130 (2006).
152. Covault, J., Gelernter, J. & Kranzler, H. Association study of cannabinoid receptor gene (CNR1) alleles and drug dependence. *Mol. Psychiatry* **6**, 501–502 (2001).
153. Benyamina, A., Kebir, O., Blecha, L., Reynaud, M. & Krebs, M. O. CNR1 gene polymorphisms in addictive disorders: a systematic review and a meta-analysis. *Addict. Biol.* **16**, 1–6 (2011).
154. Schmidt, L. G. *et al.* Association of a CB1 cannabinoid receptor gene (CNR1) polymorphism with severe alcohol dependence. *Drug Alcohol Depend.* **65**, 221–224 (2002).
155. Ehlers, C. L., Slutske, W. S., Lind, P. A. & Wilhelmsen, K. C. Association between single nucleotide polymorphisms in the cannabinoid receptor gene (CNR1) and impulsivity in southwest California Indians. *Twin Res. Hum. Genet.* **10**, 805–811 (2007).
156. Proudnikov, D. *et al.* Association of polymorphisms of the cannabinoid receptor (CNR1) and fatty acid amide hydrolase (FAAH) genes with heroin addiction: impact of long repeats of CNR1. *Pharmacogenomics J.* **10**, 232–242 (2010).

157. Li, M. J. *et al.* GWASdb: a database for human genetic variants identified by genome-wide association studies. *Nucleic Acids Res.* **40**, D1047–D1054 (2012).
158. van den Wildenberg, E., Janssen, R. G., Hutchison, K. E., van Breukelen, G. J. & Wiers, R. W. Polymorphisms of the dopamine D4 receptor gene (*DRD4 VNTR*) and cannabinoid CB1 receptor gene (*CNR1*) are not strongly related to cue-reactivity after alcohol exposure. *Addict. Biol.* **12**, 210–220 (2007).
159. Haughey, H. M., Marshall, E., Schacht, J. P., Louis, A. & Hutchison, K. E. Marijuana withdrawal and craving: influence of the cannabinoid receptor 1 (*CNR1*) and fatty acid amide hydrolase (*FAAH*) genes. *Addiction* **103**, 1678–1686 (2008).
160. Filbey, F. M., Schacht, J. P., Myers, U. S., Chavez, R. S. & Hutchison, K. E. Individual and additive effects of the *CNR1* and *FAAH* genes on brain response to marijuana cues. *Neuropsychopharmacology* **35**, 967–975 (2010).
Leverages previous investigations to provide insights on *CNR1* and *FAAH* gene interaction in association with *in vivo* neural reactivity in the reward system to marijuana-related cues. The work emphasizes the additive genetic influence on cue reactivity as an intermediate endophenotype in cannabis abuse.
161. Hutchison, K. E. *et al.* The incentive salience of alcohol: translating the effects of genetic variant in *CNR1*. *Arch. Gen. Psychiatry* **65**, 841–850 (2008).
162. Agrawal, A. *et al.* Evidence for association between polymorphisms in the cannabinoid receptor 1 (*CNR1*) gene and cannabis dependence. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **150B**, 736–740 (2009).
163. Hopfer, C. J. *et al.* Cannabis receptor haplotype associated with fewer cannabis dependence symptoms in adolescents. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **141B**, 895–901 (2006).
164. Hartman, C. A. *et al.* The association between cannabinoid receptor 1 gene (*CNR1*) and cannabis dependence symptoms in adolescents and young adults. *Drug Alcohol Depend.* **104**, 11–16 (2009).
165. Herman, A. I., Kranzler, H. R., Cubells, J. F., Gelernter, J. & Covault, J. Association study of the *CNR1* gene exon 3 alternative promoter region polymorphisms and substance dependence. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **141B**, 499–503 (2006).
166. Sipe, J. C., Chiang, K., Gerber, A. L., Beutler, E. & Cravatt, B. F. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. *Proc. Natl Acad. Sci. USA* **99**, 8394–8399 (2002).
167. Chiang, K. P., Gerber, A. L., Sipe, J. C. & Cravatt, B. F. Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. *Hum. Mol. Genet.* **13**, 2113–2119 (2004).
168. Tyndale, R. F., Payne, J. I., Gerber, A. L. & Sipe, J. C. The fatty acid amide hydrolase C385A (P129T) missense variant in cannabis users: studies of drug use and dependence in Caucasians. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **144B**, 660–666 (2007).
169. Buhler, K. M. *et al.* Risky alcohol consumption in young people is associated with the fatty acid amide hydrolase gene polymorphism C385A and affective rating of drug pictures. *Mol. Genet. Genomics* **289**, 279–289 (2014).
170. Hariri, A. R. *et al.* Divergent effects of genetic variation in endocannabinoid signaling on human threat- and reward-related brain function. *Biol. Psychiatry* **66**, 9–16 (2009).
171. Glahn, D. C., Lovallo, W. R. & Fox, P. T. Reduced amygdala activation in young adults at high risk of alcoholism: studies from the Oklahoma family health patterns project. *Biol. Psychiatry* **61**, 1306–1309 (2007).
172. Maccarrone, M., Guzman, M., Mackie, K., Doherty, P. & Harkany, T. Programming of neural cells by (endo) cannabinoids: from physiological rules to emerging therapies. *Nat. Rev. Neurosci.* **15**, 786–801 (2014).
173. Hurd, Y. L., Michaelides, M., Miller, M. L. & Jutras-Aswad, D. Trajectory of adolescent cannabis use on addiction vulnerability. *Neuropharmacology* **76**, 416–424 (2014).
174. Calvigioli, D., Hurd, Y. L., Harkany, T. & Keimpema, E. Neuronal substrates and functional consequences of prenatal cannabis exposure. *Eur. Child Adolesc. Psychiatry* **23**, 931–941 (2014).
175. Gorzalka, B. B. & Dang, S. S. Mini-review: endocannabinoids and gonadal hormones: bidirectional interactions in physiology and behavior. *Endocrinology* **153**, 1016–1024 (2012).
176. Stoving, R. K. *et al.* Leptin, ghrelin, and endocannabinoids: potential therapeutic targets in anorexia nervosa. *J. Psychiatr. Res.* **43**, 671–679 (2009).
177. Monteleone, P. *et al.* Hedonic eating is associated with increased peripheral levels of ghrelin and the endocannabinoid 2-arachidonoyl-glycerol in healthy humans: a pilot study. *J. Clin. Endocrinol. Metab.* **97**, E917–E924 (2012).
178. Monteleone, P. *et al.* Blood levels of the endocannabinoid anandamide are increased in anorexia nervosa and in binge-eating disorder, but not in bulimia nervosa. *Neuropsychopharmacology* **30**, 1216–1221 (2005).
179. Siegfried, Z. *et al.* Association study of cannabinoid receptor gene (*CNR1*) alleles and anorexia nervosa: differences between restricting and binge/purging subtypes. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **125B**, 126–130 (2004).
180. Muller, T. D. *et al.* Lack of association of genetic variants in genes of the endocannabinoid system with anorexia nervosa. *Child Adolesc. Psychiatry Ment. Health* **2**, 33 (2008).
181. Monteleone, P. *et al.* Association of *CNR1* and *FAAH* endocannabinoid gene polymorphisms with anorexia nervosa and bulimia nervosa: evidence for synergistic effects. *Genes Brain Behav.* **8**, 728–732 (2009).
182. Sipe, J. C., Waalen, J., Gerber, A. & Beutler, E. Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (*FAAH*). *Int. J. Obes.* **29**, 755–759 (2005).
183. Frieeling, H. *et al.* Elevated cannabinoid 1 receptor mRNA is linked to eating disorder related behavior and attitudes in females with eating disorders. *Psychoneuroendocrinology* **34**, 620–624 (2009).
184. Gerard, N., Pieters, G., Goffin, K., Bormans, G. & Van Laere, K. Brain type 1 cannabinoid receptor availability in patients with anorexia and bulimia nervosa. *Biol. Psychiatry* **70**, 777–784 (2011).
185. Ishiguro, H. *et al.* A nonsynonymous polymorphism in cannabinoid CB2 receptor gene is associated with eating disorders in humans and food intake is modified in mice by its ligands. *Synapse* **64**, 92–96 (2010).
186. Verty, A. N., McGregor, I. S. & Mallet, P. E. Paraventricular hypothalamic CB₁ cannabinoid receptors are involved in the feeding stimulatory effects of Δ^9 -tetrahydrocannabinol. *Neuropharmacology* **49**, 1101–1109 (2005).
187. Lewis, D. Y. & Brett, R. R. Activity-based anorexia in C57/BL6 mice: effects of the phytocannabinoid, Δ^9 -tetrahydrocannabinol (THC) and the anandamide analogue, OMDM-2. *Eur. Neuropharmacol.* **20**, 622–631 (2010).
188. Andries, A., Frystyk, J., Flyvbjerg, A. & Stoving, R. K. Dronabinol in severe, enduring anorexia nervosa: a randomized controlled trial. *Int. J. Eat. Disord.* **47**, 18–23 (2014).
189. Pataky, Z. *et al.* Efficacy of rimonabant in obese patients with binge eating disorder. *Exp. Clin. Endocrinol. Diabetes* **121**, 20–26 (2013).
190. Scherma, M. *et al.* Pharmacological modulation of the endocannabinoid signalling alters binge-type eating behaviour in female rats. *Br. J. Pharmacol.* **169**, 820–833 (2013).
191. Cottone, P. *et al.* CRF system recruitment mediates dark side of compulsive eating. *Proc. Natl Acad. Sci. USA* **106**, 20016–20020 (2009).
192. Koob, G. F. The role of CRF and CRF-related peptides in the dark side of addiction. *Brain Res.* **1314**, 3–14 (2010).
193. Blasio, A. *et al.* Rimonabant precipitates anxiety in rats withdrawn from palatable food: role of the central amygdala. *Neuropsychopharmacology* **38**, 2498–2507 (2013).
194. D'Addario, C., Di Francesco, A., Pucci, M., Finazzi Agro, A. & Maccarrone, M. Epigenetic mechanisms and endocannabinoid signalling. *FEBS J.* **280**, 1905–1917 (2013).
195. Franklin, T. B. *et al.* Epigenetic transmission of the impact of early stress across generations. *Biol. Psychiatry* **68**, 408–415 (2010).
196. Rotter, A. *et al.* CB1 and CB2 receptor expression and promoter methylation in patients with cannabis dependence. *Eur. Addict. Res.* **19**, 13–20 (2013).
The first study to report epigenetic differences in *CNR1*, demonstrating alteration of DNA methylation status (in peripheral blood cells) correlated with CB1R expression.
197. DiNieri, J. A. *et al.* Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. *Biol. Psychiatry* **70**, 763–769 (2011).
198. Tomasiwicz, H. C. *et al.* Proenkephalin mediates the enduring effects of adolescent cannabis exposure associated with adult opiate vulnerability. *Biol. Psychiatry* **72**, 803–810 (2012).
199. Stringer, R. L., Laufer, B. I., Kleiber, M. L. & Singh, S. M. Reduced expression of *brain cannabinoid receptor 1 (Cnr1)* is coupled with an increased complementary micro-RNA (*miR-26b*) in a mouse model of fetal alcohol spectrum disorders. *Clin. Epigenetics* **5**, 14 (2013).
200. Matyas, F. *et al.* Identification of the action of 2-arachidonoyl-glycerol synthesis and sites imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. *Neuropharmacology* **54**, 95–107 (2008).
201. Suárez, J. *et al.* Distribution of diacylglycerol lipase alpha, an endocannabinoid synthesizing enzyme, in the rat forebrain. *Neuroscience* **192**, 112–131 (2011).
202. Egertova, M., Simon, G. M., Cravatt, B. F. & Elphick, M. R. Localization of N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) expression in mouse brain: a new perspective on N-acyl ethanolamines as neural signaling molecules. *J. Comp. Neurol.* **506**, 604–615 (2008).
203. Egertova, M. & Elphick, M. R. Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB₁. *J. Comp. Neurol.* **422**, 159–171 (2000).
204. Matyas, F. *et al.* Subcellular localization of type 1 cannabinoid receptors in the rat basal ganglia. *Neuroscience* **137**, 357–361 (2006).
205. Lecca, S., Melis, M., Luchicchi, A., Muntoni, A. L. & Pistis, M. Inhibitory inputs from rostromedial tegmental neurons regulate spontaneous activity of midbrain dopamine cells and their responses to drugs of abuse. *Neuropsychopharmacology* **37**, 1164–1176 (2012).
206. Riegel, A. C. & Lupica, C. R. Independent presynaptic and postsynaptic mechanisms regulate endocannabinoid signaling at multiple synapses in the ventral tegmental area. *J. Neurosci.* **24**, 11070–11078 (2004).
Describes the presynaptic and postsynaptic cellular mechanisms that control eCB release and regulation of mesolimbic (VTA) DA-neuron activity via the retrograde activation of presynaptic CB1Rs. This work set the model of eCB–DA interactions, in which increased DA-neuron burst firing is mediated through eCB inhibition of VTA GABA cells (DSI), thus leading to disinhibition of midbrain DA neurons.
207. Kortleven, C., Fasano, C., Thibault, D., Lacaille, J. C. & Trudeau, L. E. The endocannabinoid 2-arachidonoyl-glycerol inhibits long-term potentiation of glutamatergic synapses onto ventral tegmental area dopamine neurons in mice. *Eur. J. Neurosci.* **33**, 1751–1760 (2011).
208. Marinelli, S. *et al.* N-arachidonoyl-dopamine tunes synaptic transmission onto dopaminergic neurons by activating both cannabinoid and vanilloid receptors. *Neuropsychopharmacology* **32**, 298–308 (2007).
209. Kravitz, A. V., Tye, L. D. & Kreitzer, A. C. Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nat. Neurosci.* **15**, 816–818 (2012).
210. Stuber, G. D. *et al.* Reward-predictive cues enhance excitatory synaptic strength onto midbrain dopamine neurons. *Science* **321**, 1690–1692 (2008).
211. Robbe, D., Alonso, G., Duchamp, F., Bockaert, J. & Manzoni, O. J. Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. *J. Neurosci.* **21**, 109–116 (2001).
212. Grueter, B. A., Brasnjo, G. & Malenka, R. C. Postsynaptic TRPV1 triggers cell type-specific long-term depression in the nucleus accumbens. *Nat. Neurosci.* **13**, 1519–1525 (2010).
213. Giuffrida, A. *et al.* Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. *Nat. Neurosci.* **2**, 358–363 (1999).
Provides the first *in vivo* evidence for interactions between the striatal DA system and eCB formation, which have substantial implications for several pathologies, including addiction and movement disorders.
214. Winters, B. D. *et al.* Cannabinoid receptor 1-expressing neurons in the nucleus accumbens. *Proc. Natl Acad. Sci. USA* **109**, E2717–E2725 (2012).

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Competing interests statement

The authors declare no competing interests.