

Review

THC and CBD: Similarities and differences between siblings

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SUMMARY

Δ^9 -tetrahydrocannabinol (THC) and its sibling, cannabidiol (CBD), are produced by the same *Cannabis* plant and have similar chemical structures but differ dramatically in their mechanisms of action and effects on brain functions. Both THC and CBD exhibit promising therapeutic properties; however, impairments and increased incidence of mental health diseases are associated with acute and chronic THC use, respectively, and significant side effects are associated with chronic use of high-dose CBD. This review covers recent molecular and preclinical discoveries concerning the distinct mechanisms of action and bioactivities of THC and CBD and their impact on human behavior and diseases. These discoveries provide a foundation for the development of cannabinoid-based therapeutics for multiple devastating diseases and to assure their safe use in the growing legal market of *Cannabis*-based products.

INTRODUCTION

The *Cannabis* plant synthesizes phytocannabinoids (phyto-CB), including the bioactive compounds THC and cannabidiol (CBD) (Figure 1). THC is often referred to as the principal “psychotropic” compound produced by *Cannabis* and CBD as the principal “non-psychotropic” compound. Since both compounds influence brain functions, this simple distinction is not valid, and this review addresses the effects of both. The recent legalization of medical and adult use of products that contain phyto-CBs by several countries, including the United States, Canada, and the Netherlands, represents a historical shift in policy that was led by patient advocates who use *Cannabis* products medicinally and by *Cannabis* industry advocates. Both groups sought to encourage a legal market that could improve well-being and reduce the use of other harmful substances (for example opioids).^{1,2} A milestone in the acceptance of CBD as a therapeutic modality was its Food and Drug Administration (FDA) approval in the treatment of refractory epilepsies. Recent studies suggest promising therapeutic indications for other neurologic diseases where CBD also modulates neuronal function.

Concomitantly, public health professionals have expressed concerns about the increasing acceptance of a substance with significant side effects, especially THC-associated mental health diseases.^{1,3} This concern is heightened by the escalation in THC concentrations found in *Cannabis* plants attributable to an increased understanding of *Cannabis* genetics, improved cultivation methods, and optimization of extraction yields (Box 1).⁴ Taking Washington state between October 2014 and September 2016 as an example, the average levels of THC in extracts reached 75%–80%, which is 3-fold more than *Cannabis* flowers (20.6%) during the same period.^{5,6} Furthermore, although traditional *Cannabis* flowers still accounted for most of the sales in Washington state at that time (i.e., 66.6%), the market share of THC extracts for inhala-

tion increased by 150%.⁵ Public health professionals in the United States have also noted the increasing perception of THC-based products as “safe” recreational drugs, particularly in 16% of individuals aged 12–17 years and in 52% of individuals aged 18–25 years (National Institute for Drug Abuse [NIDA], News release in 8/22/22). These alarming statistics indicate increased use of products that contain high levels of THC, a scenario that may have important health consequences, particularly on brain function and behavior in vulnerable individuals such as adolescents.

The research summarized in this review is discussed in the context of the known differences in the pharmacodynamic (PD) and pharmacokinetic (PK) profiles of THC and CBD. The body of work reviewed here provides a foundation for translating research into safe therapies for a variety of diseases and conditions.

PHARMACOKINETICS OF THC: DIFFERENCES AMONG ROUTES OF ADMINISTRATION

Humans administer THC-containing products by smoking, vaporizing, and dabbing (a slang term that refers to the process of consuming THC products that come in a variety of textures, including resin), as well as by drinking and eating.^{6,9} When measuring THC bioactivity, studies need to indicate (1) the route of administrations, (2) how much THC was administered, and (3) the regimen of THC use (e.g., times a day, a week, or a month). Below is our current understanding of the PK profile of THC in humans and rodents, accounting for those three factors. The PK profile of a drug depends on four key physiological events: absorption, distribution, metabolism, and excretion, typically referred to as ADME. Thus, our understanding of how the drug's concentration changes in selected tissues over time combined with knowledge of how that might correlate with the drug's activity at

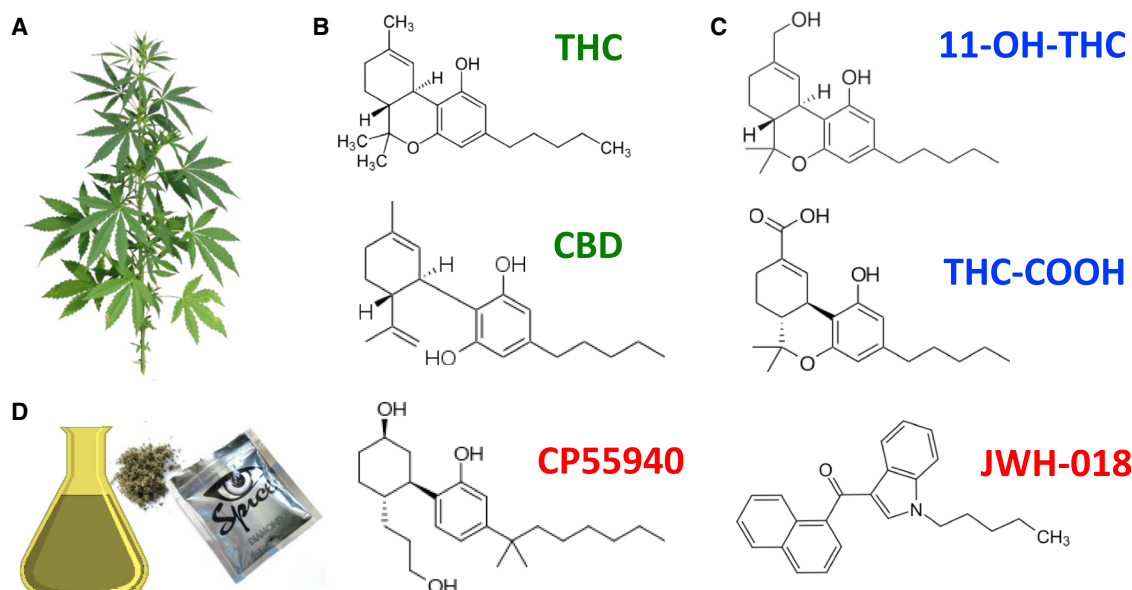


Figure 1. Cannabinoid compounds

(A) Cannabis plant.

(B–C) (B) Phytocannabinoids THC and CBD (green) produced by the plant, (C) THC metabolites 11-OH-THC and THC-COOH (blue) produced by P450 enzymes expressed in liver and brain.

(D) Artificial cannabinoids, CP55940 and JWH-018 (active ingredient in the illegal market product, “spice”) (red).

selected protein targets (i.e., its PD) provides a clearer picture of its *in vivo* bioactivity.

THC PK in humans

Inhalation of *Cannabis*-based cigarettes has historically been the most common route of THC administration by humans. It leads to rapid THC absorption that peaks within minutes in blood and is not subject to first-pass liver metabolism.¹⁰ Considering that *Cannabis*-based cigarette currently average $\approx 20\%$ of THC/total weight and that a cigarette weigh ≈ 1 g, then a full cigarette contains ≈ 200 mg THC.⁶ Thus, a human adult of ≈ 70 Kg who smokes a 1/4 of a cigarette absorbs ≈ 0.3 mg/kg of THC (considering $\approx 50\%$ pyrolytic loss because of burning the product). Human PK studies show that one inhalation of THC (0.3 mg/kg) results in vastly variable levels of THC in blood within the initial minutes, depending on the individual: 1.6–160 ng/mL of THC (≈ 5 –500 nM).¹¹ The hydrophobic nature of THC allows its distribution into most tissues, including the brain.¹² Thus, THC passes the blood-brain barrier, briefly accumulates in brain parenchyma, and then slowly declines because of THC metabolism and excretion. THC undergoes extensive liver metabolism, and its clearance is restricted by fatty acid binding proteins (FABPs).¹³ Of note, although over 80 metabolites of THC have been identified, only a handful remain bioactive, including THC’s first metabolite 11-OH-THC (more potent than Δ^9 -THC) and 11-nor- Δ^9 -THC-9-carboxylic acid (less potent than Δ^9 -THC).¹⁴

Humans are increasingly administering THC through eatables and drinks. In contrast to inhalation, oral THC absorption is slower, and maximum THC concentrations are reached 1–3 h after dosing.¹⁵ For example, oral consumption of 15 mg THC

(≈ 200 μ g/kg) results in ≈ 9 ng of THC/mL of blood (≈ 30 nM) after 3 h.¹⁶ Considering the current average amount of THC in edibles (10 mg/serving), its oral consumption by a human adult will result in ≈ 20 nM of THC in blood after 3 h. Orally administered THC and its bioactive metabolites decline slowly (half-life 20–60 h for THC), suggesting prolonged exposure and lingering bioactivity even after a single ingestion.¹⁶ Thus, the bioactivity of THC fluctuates depending on its route of administration, rate and extent of absorption, metabolism, and excretion, all of which can significantly vary between individuals (Figures 2A and 2B).¹²

THC PK in rodents

As a preclinical model system for PK studies, rodents not only provide important information but also have key limitations, including a very active liver metabolism compared with humans. The PK and bioactivity of THC in rodents has been studied when administered by i.p., i.v., and s.c. injections, as well as by smoke exposure and oral gavage, routes of administration that allow for precise dosing yet are not used by humans.^{9,17,18} For example, i.p. injections into adult mice with THC (5 mg/kg) result in 600 pmol/mL of THC (≈ 2 nM) in blood within 15–30 min and 1,000 pmol/g of THC in brain (≈ 3 nM) after 2 h.^{19,20} However, it is important to emphasize that a portion of the THC injected via i.p. will undergo first-pass liver metabolism and thus not all the injected THC will reach the brain. Obvious limitations of injections, smoke exposure, and oral gavage are the absence of self-administration and the mild stress response by rodents from administration procedures.

Oral self-administration of THC by rodents is easily achieved when formulating THC in either gelatin or dough, and

Box 1. Inheritance of cannabinoid compounds synthesized by the plant**THC CONCENTRATION IN PLANT**

Best explained by both additive and dominance composite genetic effects.

CBD CONCENTRATION IN PLANT

Best explained by cytoplasmic genomes and additive genes.^{7,8}

ENZYMES THAT SYNTHESIZE THC AND CBD

Single-nucleotide polymorphisms in the coding regions determine plant chemotype.⁷

CURRENT UNDERSTANDING

Both genetic effects and cytogenetic contributions influence the THC:CBD ratios produced by the *Cannabis* plant.⁸

OUTCOME

Facilitated effective crop breeding to select cultivars that produce defined amounts of THC and CBD.

consumption results in THC brain concentrations that are bioactive.^{21–23} For example, consumption of 2–3 mg THC-gelatin during 1 h results in 2–4 ng/mL THC in blood (\approx 6–12 nM).²¹ Of note, rodents self-administer less gelatin containing high-dose THC compared with control gelatin, suggesting an avoidance of the taste/odor of THC.²¹ Inhalation of THC by rodents involves either burning *Cannabis* cigarettes or vaporizing THC extract in an airtight environment similar to an operant chamber.^{20,24,25} THC vapor self-administration by rats is achieved by training them to nose poke for the vapor, which leads to increased motivation to take more THC vapor.²⁶ For example, 30 min inhalation exposure of 25 mg/mL results in 40 pmol/mL (\approx 30 nM) in blood that then rapidly decays.²⁶

THC is metabolized by CYP2C3 and CYP3A4, P450 enzymes expressed in liver and brain tissues, which produce similar metabolites in rodents and humans (Figures 2C and 3A).¹² When reaching the brain, THC rapidly crosses the blood-brain barrier via ABC transporters P-glycoprotein and breast cancer resistance protein.^{27–29} A recent study showed an association between an ABCB1 polymorphism in humans and increased THC metabolism and frequency of use; however, there was no difference in the self-reported subjective high associated with smoking THC (12.5% in a cigarette), suggesting a nonlinear relation between THC metabolism and subjective high.³⁰ In summary, the development of experimental approaches that better recapitulate human use of THC-containing products, combined with our growing understanding of the molecular mechanisms that control the overall PK profile of THC in rodents, provide initial mechanistic understanding of the link between the PK profile of THC and its bioactivity.

THC PHARMACODYNAMICS: MODULATION OF MULTIPLE TARGETS

THC modulates that activity of multiple G protein-coupled receptors (GPCRs) and ion channels as shown by cell culture studies,

and most of these targets mediate THC's bioactivity *in vivo* as demonstrated by fully blocking THC's bioactivity in rodent model systems using genetic deletion and/or antagonism. In this section, emphasis is on the concentrations of THC required to modulate these targets, especially concentrations of THC in the brain.

Cannabinoid receptor 1 (CB1R)

This GPCR was the first receptor shown to be modulated by THC and is the target that THC activates with the highest potency (\approx 30 nM) (Figure 4A).³¹ CB₁R mediates THC's psychotropic effects in humans as shown with the CB₁R antagonist, SR141617.³² CB₁R is involved in cell division and neuronal path-finding during development and remains expressed by most neuronal and glial cell subtypes in the adult brain, although at remarkably different expression levels depending on the cell type. For example, CB₁R is expressed at high levels by GABAergic neurons, at intermediate levels by glutamatergic neurons, and at low levels by microglia.³³ This receptor signals through G_{i/o} and β -arrestin and sometimes through G_s in neurons, microglia, and oligodendrocytes, and G_q in astrocytes (Figure 3B).^{34,35} However, coupling of CB₁R to different signaling mechanisms may vary with overexpression in heterologous systems.³⁶ CB₁R interacts with cytosolic CRIP1a and BiP proteins, and this interaction controls signaling biased toward second messenger pathways in a neuron population-selective manner.^{37,38} THC activates CB₁R as a partial agonist and modulates neuronal functions by reducing presynaptic neurotransmitter release (e.g., glutamate, GABA, and acetylcholine), inducing synaptic plasticity and adjusting energy metabolism and cell phenotype through changes in gene expression.³⁹ CB₁R expressed by astrocytes, oligodendrocytes, and microglia controls energy metabolism and cell phenotype.^{40–42} Thus, THC influences the function of most neuronal and glial cells in the brain by modulating their CB₁R cell-specific signaling.

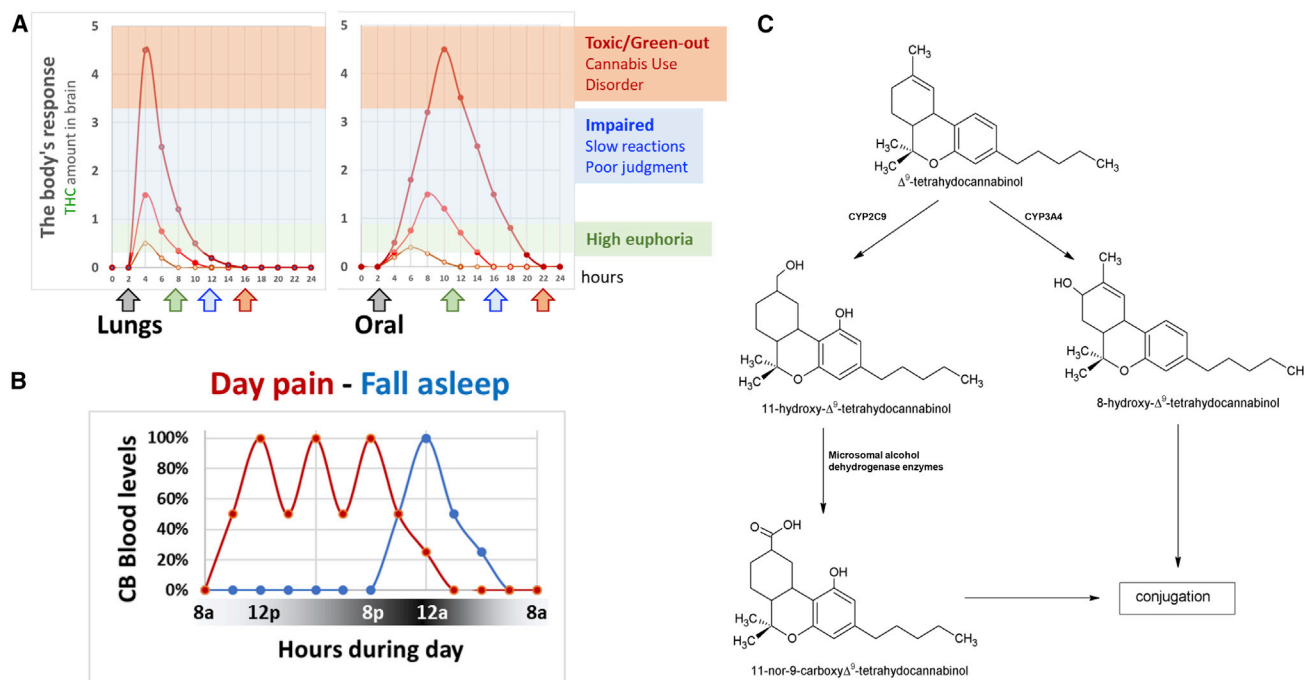


Figure 2. THC metabolism, PK, bioactivity, and regimens

(A) Model examples of the PK profiles of THC when inhaled (lungs) or consumed (oral). Examples of resulting behavioral responses associated with low-dose THC producing the “high”/euphoria (green arrow), higher-dose THC that impairs behavior such as reaction time and judgment (blue arrow), and even higher-dose THC (red arrow) that might lead to Cannabis use disorder in vulnerable populations and can be toxic and associated with physiological and psychological panic attacks (commonly referred to as “to green-out”; symptoms include sweating, nausea, heart palpitations, hypervigilance, paranoia, and the fear or feeling that you might be dying or about to die).

(B) Model examples of the PK profile of THC when used to treat pain during the day using several administrations (red line) or to fall asleep with one administration (blue line).

(C) Schematics of THC metabolism by separate enzymes to initial metabolites, which then undergo conjugation as further chemical modification. THC is either metabolized by CYP2C9 that produces 11-OH-THC, which is then metabolized by microsomal alcohol dehydrogenase, or CYP3A4 that produces 8-OH-THC. Conjugation represents the next metabolic step.

Cannabinoid receptor 2 (CB₂R)

CB₂R is expressed at low levels in the healthy brain and predominantly by endothelial cells forming the blood-brain barrier.^{43,44} Its expression is greatly upregulated under select pathological states, (e.g., activated microglia in the mouse model of experimental autoimmune encephalomyelitis).⁴⁵ Although both genetic and pharmacological evidence indicate that CB₂R directly regulates neuronal functions, its expression by neurons remains inconclusive because of the absence of reagents such as selective antibodies.⁴⁶ CB₂R signals through G_{i/o} and β -arrestin in microglia and oligodendrocytes and sometimes through G_q in endothelial cells.^{40,47} Thus, THC's bioactivity mediated by CB₂R includes glial cell activation and endothelial cell function, although it is undetermined whether modulation of neurons is direct or indirect through these cell types (Figure 4A).

Endocannabinoid-dependent modulation of CB₁R/CB₂R

CB₁R and CB₂R are endogenously activated by two signaling lipids, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), that are produced and inactivated by neuronal and glial cells⁴⁸ (Figure 5). These endocannabinoids (eCB) are produced on demand in response to select stimuli that increase cellular activity (typically by increasing the calcium-dependent activity of membrane-associated lipases).⁴⁹ AEA is mainly produced by

N-acetylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD), whereas 2-AG is mainly produced by diacyl glycerol lipase (DAGL).⁵⁰ Thus, AEA and 2-AG are released from plasma membranes and act in an autocrine and paracrine manner to modulate CB₁R/CB₂R signaling.

The amount of AEA and 2-AG that reach and activate CB₁R/CB₂R is regulated by at least five mechanisms: (1) enzymes that produce eCBs, (2) eCB transport from their synthesis to their target receptors, which involves FABPs, (3) the number of functional CB receptors at the membrane and their biased signaling, (4) the clearance of eCBs by uptake through the plasma membrane, and (5) eCB inactivation by enzymatic hydrolysis. Specifically, presynaptic fatty acid amide hydrolase (FAAH) hydrolyzes AEA, whereas presynaptic monoacylglycerol lipase (MAGL) and post-synaptic α/β -hydrolase domain 6 (ABHD6) hydrolyze 2-AG.^{51,52} Importantly, these proteins are expressed by neuronal, glial, and endothelial cells; their contribution in controlling eCB signaling has recently been reviewed.⁵³ Thus, eCB signaling encompasses CB₁R/CB₂R, AEA/2-AG, and the enzymes that produce and inactivate these two main eCBs. eCBs exhibit different potencies at CB₁R/CB₂R, and provide parallel signaling systems to regulate multiple brain functions.

The PD differences between 2-AG and AEA include their potency and efficacy at CB₁R/CB₂R; 2-AG acts as a low

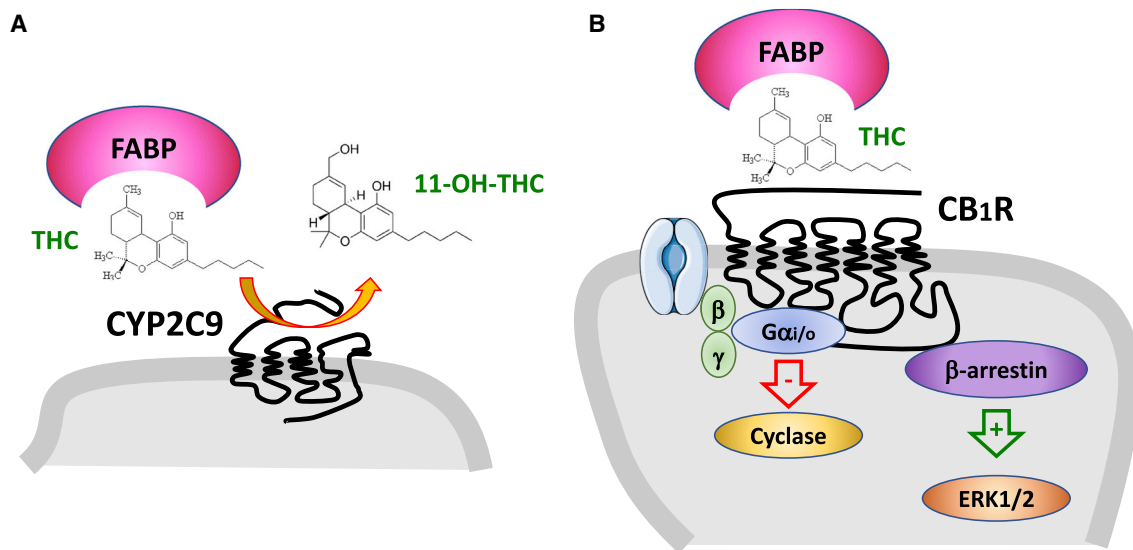


Figure 3. THC inactivation by CYP2C and biased signaling at CB₁R

(A) THC is metabolized to 11-OH-THC by the P450 enzyme CYP2C9. Fatty acid binding proteins (FABPs) assist THC in being metabolized.

(B) THC activates CB₁R that couple to Gα_{i/o} proteins that inhibit adenylyl cyclase, Gβγ proteins that regulate ion channels and β-arrestin that regulates kinase signaling. Different agonist will often preferentially modulate one of these signaling pathway. FABPs assist THC in activating CB₁R.

potency full agonist compared with the high potency and partial agonist AEA. Considering that THC has a higher potency at CB₁R/CB₂R and activates these GPCRs as partial agonists/antagonists, respectively, this phyto-CB may both partially activate free CB₁R/CB₂R and compete for eCB activity at CB₁R/CB₂R.⁵⁴ Thus, THC more likely mimics AEA's bioactivity and reduces 2-AG's bioactivity. A notable difference is that THC modulates CB₁R/CB₂R signaling in the entire brain compared with the localized, activity-dependent, increases in AEA/2-AG levels and ensuing activation of CB₁R/CB₂R. Furthermore, CB₁R, and probably CB₂R, represents molecular hubs/coincidence detectors that respond to several endogenous signaling molecules through allosteric binding sites modulating their activity.^{55,56} Specifically, an allosteric binding site is a distinct protein domain from the orthosteric site that binds the primary endogenously produced ligand and modulates the protein's activity. Thus, there are both positive and negative allosteric modulators. A remarkable example is pregnenolone, which acts as a negative allosteric modulator of CB₁R. As pregnenolone synthesis is CB₁R dependent, this mechanism represents an endogenous negative feedback loop mechanism thought to protect the brain from CB₁R overactivation.⁵⁷ This allosteric modulation site on CB₁R represents a promising mechanism to target and treat several devastating neurological and psychiatric diseases (see below).⁵⁸

Glycine receptor

The currents mediated by this ligand-gated chloride ion channels are enhanced by AEA and THC (EC_{50} s = 0.1–1 μ M and 3 μ M, respectively) and are thought to act through a positive allosteric mechanism (Figure 3).^{59,60} Accordingly, the commonly studied THC-induced analgesia is absent in α 3GlyR knockout mice and intact in CB₁R/CB₂R knockout mice.⁶¹

This research showed that a commonly studied THC bioactivity, here analgesia, may also be partially mediated by glycine receptors and that the selective targeting of this mechanism may produce specific analgesic benefit distinct from the analgesia mediated through CB₁R and CB₂R (see below). These results also uncover a new coincidence detector mechanism mediated by the synchronized release of glycine and AEA onto cells expressing glycine receptors. Such a coincidence detector mechanism likely contributes to locally inhibiting neuronal activity (Figure 4A).

Other possible targets

THC modulates the activity of at least two additional GPCRs when tested in cells in culture. THC (1–5 μ M) is a partial agonist at post-synaptic GPR55 and reduces excitatory transmission (Figure 4B).^{62,63} GPR55 is endogenously activated by the lipid signaling molecule, lysophosphatidic acid, and possibly the small peptide PACAP-27.^{64,65} GPR55 couples to Gq and β-arrestin and is expressed by neurons, astrocytes, and microglia.^{66–68} Thus, by only partially activating GPR55, THC might prevent endogenous activation of GPR55 and reduce excitatory transmission. Cell culture evidence suggests that AEA decreases and THC increases 5-HT_{3A}R currents (EC_{50} s ≈ 300 nM and 30 μ M, respectively) (Figure 4B).^{69,70}

In summary, the strong evidence above shows that the nanomolar concentrations of THC reached in the brain modulate the activities of multiple proteins, including CB₁R, CB₂R, and glycine receptors (GlyR), and possibly GPR55 and 5-HT_{3A}R. This polypharmacology profile of THC suggests that selective activation of each target will not fully recapitulate THC's bioactivity and that selectively modulating a target may provide specific therapeutic benefits and avoid THC's associated psychotropic effects mediated by the orthosteric site of CB₁R (see below).

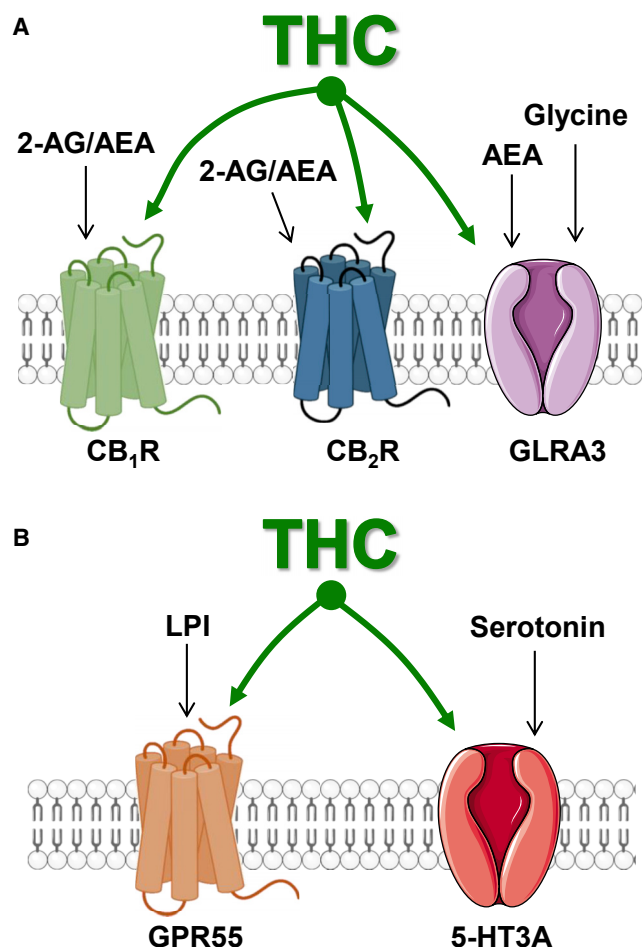


Figure 4. Molecular targets modulated by THC

(A) THC modulates CB₁R and CB₂R that are endogenously activated by 2-AG and AEA, and GLRA3 receptors that are endogenously activated by glycine, as demonstrated by *in vivo* genetic and antagonist experiments.

(B) THC modulates GPR55 that is endogenously activated by lysophosphatidyl inositol (LPI) and 5-HT_{3A} receptors that are endogenously activated by serotonin, as suggested by cell culture experiments.

THC BIOACTIVITY: FROM HUMANS TO RODENTS AND BACK

The landmark discovery of the chemical synthesis of THC in the 1960s enabled preclinical and human research on its bioactivity.⁷¹ Early studies aimed at comparing the bioactivity of injected THC versus smoked *Cannabis* in humans established that both trigger similar somatic, perceptual, and cognitive changes. Specifically, both smoking a *Cannabis* cigarette containing THC (12 mg) or injecting THC i.v. (1–6 mg) trigger a rapid succession of symptoms, starting with numbness and tingling of the extremities, light-headedness, “floating” feelings, and loss of concentration, followed by euphoria, palpitation, sweating, tremulousness, and weakness that lasts several hours.⁷² Subjects also report cognitive impairment described as difficulty in paying attention, difficulty in expressing oneself, mental confusion, and loss of sense of time (e.g., one min feels like several min)^{72,73}

(Table 1). Below are the main behavioral changes commonly measured in rodents exposed to THC, and their relevance to THC’s bioactivity in humans.

Cannabinimetic responses in rodents

Early studies outlined the broad bioactivity of THC (1–30 mg/kg, i.p.) in rodents and showed a progressive reduction in spontaneous locomotion, hypothermia, hypersensitivity to tactile and auditory stimuli, ataxia, and sedation, all of which were paralleled by increases in serotonin and noradrenalin levels in the brain.^{74,75} The first experimental approach commonly used to test the bioactivity of cannabinoids in rodents is referred to as the “classic tetrad behavior test,” which includes four easy-to-measure physiological and behavioral changes: hypothermia, hypolocomotion, analgesia, and catalepsy (Figure 6A).⁷⁶ Most, but not all, cannabinimetic responses are mediated by CB₁R expressed by select cell types, principally glutamatergic neurons and astrocytes (see below).^{77–83} Furthermore, THC bioactivity, impairing effects, and therapeutic effects differ significantly depending on the age and sex of mice. For example, THC (5 mg/kg) reduces spontaneous locomotor activity and is anxiogenic in adult mice but not adolescents and exhibits stronger bioactivity in females.^{19,26,84} These behavioral measures in rodents have evident translational value in understanding THC’s bioactivity in humans.

Oral consumption and vapor inhalation of THC can be reliably self-administered by rodents (Figure 6B). Voluntary oral self-administration of THC by rats and mice results in significant acute CB₁R-dependent cannabinimetic responses.^{21–23,85} Rats trained to nose poke for THC vapor show high rates of responding and motivation to take vapor.^{24,86} Significantly, rats and mice can reliably discriminate between vehicle and THC injections through a CB₁R-dependent mechanism (e.g., i.p. injections of THC (10 mg/kg) versus vehicle injection in a 2-lever drug discrimination test for sucrose reward).⁸⁷

THC chronic administration results in sex-specific tolerance to its bioactivity, principally because of receptor desensitization dependent on CB₁R phosphorylation by a G protein-coupled receptor kinase and CRIP1A interaction.^{38,88–90} In rodents, THC tolerance is associated with altered sleep during spontaneous cannabinoid withdrawal, a mechanism mediated by impaired dopamine function.^{91,92} Thus, rodents will self-administer THC, can discriminate its bioactivity from control treatment, and exhibit increasing tolerance to its bioactivity, providing a direct translational value to understanding THC’s bioactivity in humans.

Impairing effects triggered by THC

Impairment is defined as a state or condition associated with marked reduction in an individual’s ability to control physical and mental functions. The importance of measuring impairment is critical. Motor vehicle accidents are a leading cause of mortality across the world. Accordingly, both simulated and real driving studies that performed a double-blind, placebo-controlled testing of THC administration (20 mg in a cigarette) show a significant reduction in driving stability as measured by inappropriate line crossings.^{93,94} Can such impaired behavior be studied in rodents using simplified approaches?

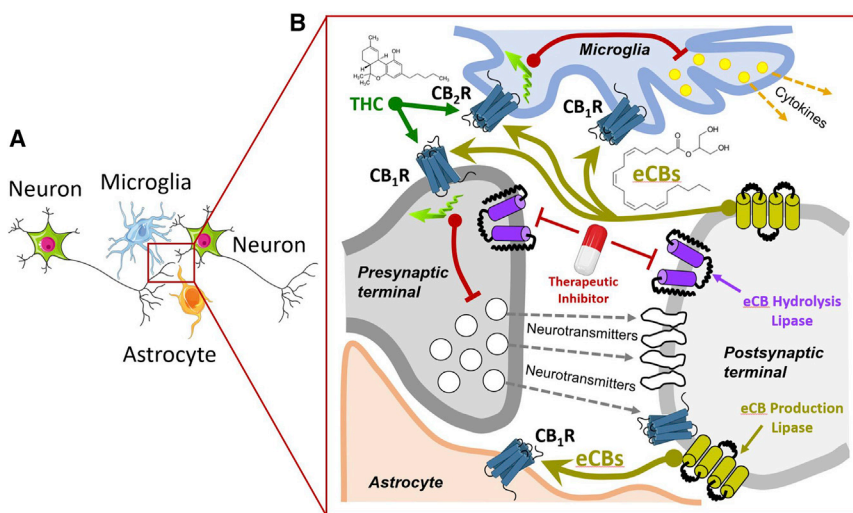


Figure 5. Endocannabinoid signaling

(A) Focus on a synapse between two neurons with adjacent microglia and an astrocyte (tripartite synapse).

(B) Presynaptic release of neurotransmitters (gray dotted arrows) stimulates post-synaptic receptors and activate lipases involved in eCB production (light green arrow). Released eCB activate pre-synaptic CB₁R and inhibit neurotransmitter release (red line), CB₁R expressed by astrocytes and regulate their energy metabolism, CB₁R/CB₂R expressed by microglia and inhibit cytokine production. THC modulates these receptors. Therapeutic inhibitors that target eCB hydrolysis result in increasing local eCB levels and activity at their targets.

Behaviors in rodents used to measure impairment from THC include locomotion, motor control, reaction time, and the startle response to sudden noise. THC affects spontaneous locomotion of mice in an open field through a CB₁R-dependent mechanism that changes with dose: a low dose increases locomotion and a high dose reduces locomotion.^{95,96} CB₁R KO mice show worse motor coordination than WT mice as measured by the Rotarod performance test.^{97,98} Impaired motor coordination is thought to result from dysfunction of the cortico-striatal neuronal circuits.⁹⁹ CB₁R is expressed at different levels in striatum: higher levels by GABAergic medium spiny neurons and parvalbumin-expressing interneurons, and lower levels by cortical neurons that project to the striatum.^{100–108} Evidence suggests that CB₁R expressed in cortico-striatal terminal and in medium spiny neurons control motor coordination skills. Accordingly, brain slice electrophysiological studies show that cannabinoids dampen cortico-striatal activity through both CB₁R and CB₁R/D2 receptor heteromers located on cortical axon terminals abutting D2 receptor-expressing medium spiny neurons.^{100,108–112} CB₁R expressed by medium spinal neurons are involved in the initial learning of motor coordination skills measured on a Rotarod.⁹⁸ Thus, rodent studies point to CB₁R expressed by the cortico-striatal pathway as playing a prominent role in the control of spontaneous locomotion and motor coordination and show that THC impairs this mechanism.

Startle is defined as a largely unconscious defensive response to sudden or threatening stimuli, such as sudden noise or sharp movement. In mice, THC (10 mg/kg) decreases the startle response as measured by the reflex response to loud acoustic stimulation via CB₁R- and 5HT_{2A}-dependent mechanisms.^{113,114} Furthermore, THC (10 mg/kg) reduces reaction time tasks in rats as measured by the trial-unique, delayed non-matching-to-location task, an index of executive function involving working memory and attention.^{113,115}

A recent human study showed that THC (36 mg, oral) impacts neuronal connectivity in prefrontal cortex (PFC), as measured by functional near infrared spectroscopy (fNIRS), a commonly used

neural signature of impairment in humans.¹¹⁶ CB₁R is expressed at different levels by distinct neuronal subpopulations in the PFC of rodents: higher levels by GABAergic projecting neurons and interneurons, and lower levels by projecting glutamatergic neurons.¹¹⁷ Activation of CB₁R in rodent PFC slice electrophysiology recording shifts the balance between excitation and inhibition toward excitation, and THC administration to mice results in a remarkable upregulation in CB₁R expression and downregulation of brain-derived neurotrophic factor (BDNF) in the PFC.^{118–120} Accordingly, the sensitive balance of glutamatergic and GABAergic transmission within the PFC is particularly vulnerable to THC that downregulates CB₁R function.^{121–123} These rodent studies enhance our understanding of a molecular mechanism associated with THC-triggered impairment that affects CB₁R signaling in the PFC and might mediate THC-induced impairment.

Acute oral consumption of THC (20 mg/kg) impairs memory functions for hours in humans.¹²⁴ This finding was validated in a double-blind trial that tested 7.5–15 mg oral THC on visual working memory.¹²⁵ In mice, THC impacts spatial reference and working memory in a sex-dependent manner.^{126,127} Mechanistically, THC disrupts network neuronal spiking patterns and temporal firing synchrony in the hippocampus, a response that is mediated through CB₁R expressed in astrocytes, PKC, and COX2 signaling in addition to hyperpolarization activated cyclic nucleotide-gated channels.^{128–133}

Thus, THC triggers a broad range of well-defined behavioral changes in rodents, some of which are considered close proxies of human behavior and cognitive tasks, including memory processing, motor coordination, and startle responses (Figures 6B and 6C).^{134–137} Importantly, the bioactivity of THC in rodents shifts depending on the dose. For example, although THC (1 mg/kg) increases food consumption and locomotion and has anxiolytic effects, higher-dose THC (5 mg/kg) reduces locomotion and produces anxiogenic and aversive effects.^{26,113,115} Such a biphasic response to THC is also apparent in humans: low doses of THC alter perception, produce euphoria, impair verbal fluency, and impair working memory, whereas higher doses increase anxiety. Rapid-onset high doses trigger schizophrenia-like symptoms in some individuals.¹³⁸ In summary, some of the cardinal behavioral impairments triggered by THC in humans

Table 1. Psychotropic bioactivity of THC in humans Examples of changes in human somatic, perceptual, and cognitive functions triggered by acute THC use

Somatic	Perceptual	Cognitive
Light-headedness	euphoria	introspective states
"Floating" feelings	loss of time sense	rapid flow of thoughts
Pulse rate increases	increased body awareness	Dreamy
Palpitation	distortions of vision	loss of concentration
Sweating	decreased hearing	disrupted memory
Tremulousness	decreased paying attention	Anxiety
Weakness	mental confusion	Incoordination
Numbness	dizziness	Sleepiness
	blurring	difficulty in thinking
	fatigue	difficulty in speaking
		difficulty in reading
		difficulty in remembering

can be studied in rodents and thus have evident translational value.

Mental health disorders associated with THC use: Epidemiology and preclinical studies

Chronic use of high amounts of THC impacts brain function and is associated with increased incidence of mental health disorders. I will highlight two predominant disorders: cannabis use disorder (CUD) and psychosis or schizophrenia.¹³⁹ The impacts of THC during pregnancy and in the elderly have been recently reviewed and are not covered here.^{12,140–142}

Based on self-reporting surveys between 2016 and 2017 across the United States, *Cannabis* dependence was the highest among young adults (16.7%).¹ The public health community is concerned that the use of high-dose THC-base products often increases during adolescence, may contribute to transitioning from experimental use to frequent use, and may increase impulsive/risky behaviors.^{3,143–146} CUD is characterized by several altered behavioral and cognitive functions, including impaired memory processing, decision-making, school/work performance, and social functioning. Chronic use of high concentration THC product is associated with progression to first CUD symptoms in adolescents.^{147,148} Adolescents with CUD also have predicted lower intelligence quotients and slower cognitive function as measured by full-scale IQ and reaction time testing.¹⁴⁹ The largest online drug survey worldwide, the Global Drug Survey, along with the US National Comorbidity Survey database, indicates that acute *Cannabis* use can trigger psychotic symptoms as defined by the occurrence of hallucinations and/or paranoia in select individuals, including young adults who regularly use high amounts of *Cannabis* and individuals diagnosed with psychosis.^{150,151} Correspondingly, i.v. injection of THC (2.5 and 5 mg) produces a broad range of transient symptoms and cognitive deficits in healthy individuals that resemble some aspects of endogenous psychoses.^{138,152} Furthermore,

daily use of *Cannabis*-based products with high THC increases the risk of developing psychotic disorders, including schizophrenia, and is related to earlier onset of symptoms compared with people who do not use *Cannabis*.^{153–156} Thus, daily use of products with high THC levels during adolescence may have a strong impact during adolescence itself and later in adulthood in some individuals and, in particular, may increase the incidence of CUD, psychosis, and schizophrenia.^{154,157}

A recent flurry of results gathered in rodents outline the impact of THC use on adolescent rodent behavior and on later adulthood behavior. Administration of THC (3 mg/kg i.p. daily for 3 weeks) to adolescent rats is associated with anxiety-like and impaired locomotor behavior in adulthood, indicated by increased repetitive and compulsive-like behaviors measured by the Nestlet shredding task.¹⁷ Confirming and extending this result, administration of THC (0.3–3 mg/kg, twice daily for 10 days) to adolescent rats is associated with anxiety-like behavior in adulthood as measured by the elevated plus maze, increased spontaneous locomotion in open field, and no change in attentional set-shifting performance.¹¹⁸ Administration of adolescent mice with THC (3–8 mg/kg, daily for 3 weeks) impairs recognition memory, working memory, and novel object recognition in adulthood.^{17,158,159} THC exposure during adolescence does not influence adult nicotine self-administration, extinction, and reinstatement nor does it influence conditioned place preference to THC itself, pre-pulse inhibition or attentional set-shifting performance in adulthood.^{118,160,161} In a rat model of anxiety-like behavior, acute intra-PFC infusions of THC produce anxiogenic effects while producing no observable impairments.¹⁶² Finally, glial cells are also involved in the adolescent brain response to THC. For example, microglia-mediated phagocytosis of newborn cells during development is controlled by eCB signaling and sculpts sex differences in juvenile rodent social play.¹⁶³ A recent study based on analyzing microglia phenotype by RNA sequencing showed that daily low-dose administration of THC to adolescent mice markedly changed microglia activation and function and impaired the stress behavior caused by social defeat in repeated resident-intruder encounters, an experimental paradigm that produces a depression-like phenotype, anxiety, and social avoidance.¹⁶⁴ Thus, the influence of THC on the developing brain appears to impact multiple neuronal and glial cell functions that affect adulthood behaviors.^{41,165}

Whether THC acts as a gateway drug, predisposing individuals to want to take "harder" drugs such as opiates or psychostimulants, has been a longstanding debate. CB₁Rs are densely expressed in brain areas involved in reward, addiction, and executive function, including the amygdala, PFC, nucleus accumbens and ventral tegmentum area (VTA) (Figure 7).¹⁶⁶ Because adolescence represents a vulnerable time of marked neuronal development, it is possible that THC use during this period changes an underlying neurophysiology that makes drugs that are already reinforcing even more rewarding. Several rodent studies proved this hypothesis correct. Adult rats show increased heroin self-administration when exposed to THC during adolescence (1.5 mg/kg, 8 injections over 21 days), a response that relies on increased pro-enkephalin expression in the nucleus accumbens.^{167–169} Adult rats also show increased cocaine self-administration when exposed to THC during

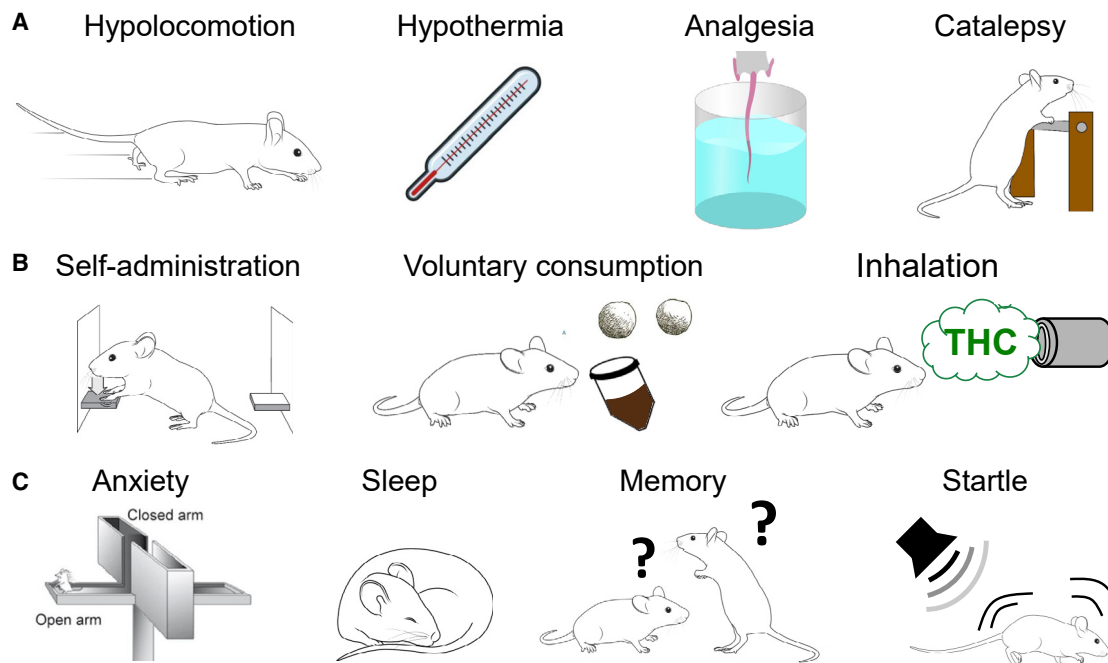


Figure 6. Examples of THC bioactivity in rodents

(A) “Classic tetrad” behaviors triggered by CB₁R agonists.

(B) Recent experimental approaches to study THC self-administration.

(C) Behavioral changes induced by THC in rodents with translational relevance to humans.

adolescence (1 mg/kg i.p., 18 days).¹⁷⁰ These effects of THC are likely mediated by CB₁R. Indeed, CB₁R is involved in the development of the rewarding properties of psychostimulants mediated by dopamine, including amphetamine and cocaine, as shown by CB₁R genetic deletion and SR1 treatment.^{171–174} The dopaminergic system that originates in the midbrain and projects to select areas of the forebrain to regulate motivated behaviors is particularly sensitive to chronic THC exposure, and its dysfunction is associated with drug addiction.^{21,175} THC exposure (2.5–10 mg/kg, daily for 10 days) during adolescence in rats affects the functionality of dopamine receptors and dysregulates glutamate receptor expression in adulthood.^{176–179} These results paint a grim picture in which THC exposure during adolescence delays the maturation of the dopamine and glutamatergic neurons and impairs their ability to control motivated and reward-triggered behaviors.

THC THERAPEUTIC INDICATIONS: FAVORABLE EVIDENCE

Despite the negatives discussed above, THC shows promising therapeutic efficacy. Considering the multiple pathways that THC modulates, an understanding how each target might be involved in THC’s therapeutic response will enable the development of selective small molecules with improved therapeutic index compared with THC. Below I outline preclinical evidence for use of THC in the treatment of pain, sleep disorders, seizures, multiple sclerosis (MS), and Huntington’s disease (HD).

Probably the original and oldest therapeutic use of THC was for its analgesic properties in chronic pain.^{180,181} We now know that acute inhalation of THC (0.5–1 mg) produces greater analgesia compared with placebo in chronic pain patients and causes only mild side effects that resolve spontaneously.¹⁸² Based on our understanding of THC’s molecular and cellular mechanisms of action, preclinical studies are optimizing this analgesic activity.^{181,183} The antinociceptive efficacy of THC in rodent models of both pathological and injury-related persistent pain are well established; in addition, the expression pattern of most of its targets in pain pathways have been precisely mapped. Furthermore, the opioid-sparing effects and antinociceptive synergy with nonopioid analgesics have been described in detail, and co-administration of heroin and THC (50 mg/mL) by inhalation produces additive effects on thermal nociception in rats.^{181,184,185} Mechanistically, oral THC (\approx 3 mg/kg) reduces neuropathic pain in mouse injury via a CB₁R and through CB₂R in the mouse model of chemotherapy-induced neuropathy without the development of tolerance.^{22,85,186} Of particular relevance for the therapeutic relevance of this mechanism, rats with nerve injury self-medicate with CB₂R small molecule agonists to attenuate a neuropathic pain state.¹⁸⁷ Thus, THC induces analgesia by interacting with at least 3 targets: CB₁R, CB₂R, and GlyR, and their relative involvement depends on the drug exposure and experimental paradigm, emphasizing that the selective targeting these molecular components involved in pain sensation represents independent therapeutic approach. Dedicated resources to the optimization of cannabinoid-based analgesics is particularly relevant to the goal of reducing the use of

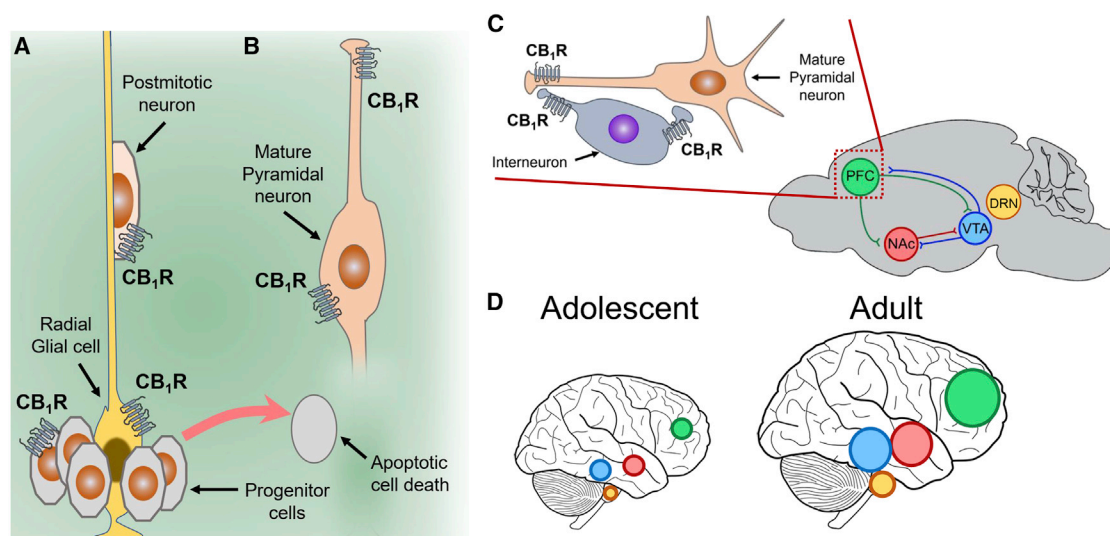


Figure 7. CB₁R-dependent impact of THC on the developing brain during adolescence

(A) CB₁R are expressed by progenitor cells and their activation by THC can trigger apoptotic cell death and impair the development of radial glial cells and postmitotic neurons.

(B) THC activation of CB₁R expressed by mature neurons affects their phenotype and neurotransmitter release.

(C) CB₁R expressed by mature pyramidal neurons and inhibitory interneurons control the excitatory/inhibitory transmission balance in several brain areas, including prefrontal cortex (PFC), nucleus accumbens (Nac), and ventral tegmentum area (VTA). THC also interacts with 5HT_{1A} receptors in the dorsal raphe nucleus (DRN).

(D) PFC, Nac, and VTA in human adolescents undergoes significant development and maturation into adulthood.

opioid-mediated analgesics that are addictive and that represent a current major health problem.¹⁸⁸

The effect of THC on sleep quality has been explored since increased sleepiness is often reported following THC use. Low-dose THC enhances subjective sleep quality, which is measured by the insomnia severity index via a clinically validated questionnaire, whereas high-dose THC increases insomnia symptoms.^{189,190} One consistent finding is the short-term benefit of THC to treat sleep apnea, most likely due to its modulatory effect on serotonin transmission in key brain areas.¹⁹¹ However, chronic use of *Cannabis* is associated with rapid habituation to its sleep inducing and enhancing properties and represents a primary reason for the lapse/relapse of using *Cannabis*.¹⁹² Specifically, abrupt *Cannabis* use cessation among heavy users impairs sleep, may sabotage attempts to quit *Cannabis* use, and raises the risk of relapse and emotional behavior (e.g., irritability and anhedonia).^{193–195} One of the most intriguing results is found in individuals suffering from post-traumatic stress disorder who experience both emotional and behavioral symptoms from previous traumatic events. Here, THC (5 mg/oral) improves subjective measures of sleep quality and reduces the frequency of nightmares in 70% of the subjects.^{196–198} Thus, THC may decrease sleep latency initially but could impair sleep quality long term, depending on the individual.^{192,195} Studies on the effect of THC on sleep in rodents show that mice exhibit altered sleep during spontaneous THC withdrawal through a CB₁R-dependent mechanism and changes in striatal dopamine signaling.^{91,199} CB₁R-KO mice spend more time awake during the dark (active) period but not during the light (rest) period, thus enhancing the day-night variation of wake-sleep hours compared with WT mice.²⁰⁰

There is also evidence that THC may be useful for the treatment of seizures, as shown in several rodent models of epilepsy. THC (1–10 mg/kg) reduces both electrically and chemically induced seizures in rats through a CB₁R-dependent mechanism.^{201,202} Accordingly, THC and CB₁R agonists reduce excitatory transmission and hippocampal overexcitation known to cause seizures.^{129,203–205} Of note, CB₁R expression is both upregulated and involved in the development of rodent models of seizures, suggesting increased therapeutic efficacy when selectively targeting this mechanism. However, considering THC's side effects, few studies followed up on the therapeutic efficacy of THC as an antiepileptic drug, and the field moved to targeting the eCB signaling and the allosteric site of CB₁R.^{206,207} For example, inhibition of MAGL increases 2-AG levels and reduces seizures through a CB₁R-dependent mechanism in chemically induced seizures in rats.^{208,209} Treatment of a rat genetic model of childhood epilepsy with the small molecule allosteric CB₁R modulators, GAT211 and GAT229, reduces *in vivo* cortical spikes and wave discharges, a measure of absence seizures that are characterized in humans by staring blankly into space for a few seconds.²¹⁰

Several clinical trials of THC for the treatment of MS showed limited therapeutic efficacy at reducing spasticity and pain.^{211,212} However, in a mouse model of MS (experimental autoimmune encephalomyelitis; EAE) activation of CB₁R expressed by neurons and CB₂R expressed by immune cells reduced disease severity.^{213–216} The therapeutic promise of targeting CB₂R is underscored by a 200-fold upregulation of its expression in microglia in the EAE mouse model.⁴⁵ Thus, preclinical studies suggest that the mild therapeutic properties of THC

will require optimization, for example by testing CB₁R/CB₂R allosteric modulators.

THC was tested therapeutically for HD as part of a clinical trial and showed no significant symptomatic effects when prescribed at 3 mg/kg administered *via* sublingual spray for 12 weeks.²¹⁷ This lack of therapeutic efficacy should be interpreted in the context of the pronounced downregulation of CB₁R expression in striatum, a predominant brain structure that undergoes neurodegeneration in HD.¹⁰³ CB₁R expression is also downregulated in multiple genetic mouse models of HD, and CB₁R genetic rescue prevents the loss of striatal synaptic contacts.^{97,218–220} THC exhibits only small therapeutic efficacy in mouse models of HD, indicating that either THC treatment requires optimization or that we need to consider targeting eCB signaling.^{221,222} For example, MAGL expressed in astrocytes controls mutant huntingtin-induced damage of striatal neurons as shown by the therapeutic efficacy of MAGL inhibition.²²¹ Remarkably, THC treatment is associated with significant neuroprotection in mouse models of HD through a CB₁R/CB₂R-independent mechanism of action; a result that is encouraging and prompts further investigations.²²³

In summary, human trials and preclinical rodent models of chronic pain suggest promising therapeutic properties of THC that would benefit from optimization, but preclinical rodent models of insomnia, epilepsy, MS, and HD suggest that THC therapy would require significant optimization, most likely focusing on non-CB₁R-dependent mechanisms.

CBD PK/PD: SIMILARITIES WITH AND DIFFERENCES FROM THC

Since the legalization of CBD-containing products by several countries, including the United States in 2018, there has been a striking increase in the human use of CBD-containing tinctures, drinks, and edibles.^{224,225} Selective cross breeding of *Cannabis* has resulted in a plant producing high levels of CBD and low levels of THC (below 0.3%).⁷ In the United States, this plant is now legally referred to as hemp. Concomitantly, peer-reviewed studies have reported behavioral and therapeutic effects of CBD in preclinical and human trials and have increased our understanding of the behavioral changes induced by CBD administered as a single agent, its mechanism of action and its therapeutic promise. Again, to measure CBD bioactivity in humans, studies need to provide information on route of administration, how much CBD was used, and its regimen of use. Our understanding of the PK profile of CBD, and the multiple protein activities that it modulates provides a clearer picture of its bioactivity *in vivo*.

CBD PK in humans and rodents

The PK profile of CBD in humans when administered by different routes of administrations has only recently been studied in detail. In humans, inhaled and oral CBD (10 mg) results in blood levels reaching maximal concentration values of 105 and 14 ng/mL, respectively (\approx 330 and 45 nM); values that are comparable with those obtained with inhaled and oral THC (10 mg).²²⁶ Oral CBD undergoes first hepatic metabolism that forms 7-COOH-CBD with a more pronounced and prolonged exposure profile than inhaled CBD.²²⁶ Whether 7-COOH-CBD remains bioactive

is unknown. The therapeutic potential of oral CBD formulations is limited by poor bioavailability and extensive first-pass hepatic metabolism. For example, following oral administration, 7-COOH-CBD blood concentration is \sim 40-fold higher than CBD.²²⁷ Significantly, inhalation as a route of administration has several advantage compared with oral—it bypasses the PK variability attributed to irregular gastrointestinal absorption and first-pass hepatic metabolism and efficiently delivers CBD into systemic circulation.²²⁷ In rodents, *i.p.* and oral gavage delivery of CBD results in a PK profile that parallels the PK profile of THC.^{19,228} Because of the similarity of its chemical structure to THC, CBD is also metabolized by CYP3A4, and thus, CBD competes for THC metabolism by this enzyme and augments THC levels by reducing its conversion to 11-OH-THC.²²⁹ Of note, the benzodiazepine, clobazam, is also metabolized by CYP3A4, and thus, CBD treatment augments the exposure of clobazam; however, this molecular interaction does not significantly affect the bioactivities of CBD and clobazam (see below).^{230,231} In summary, the PK profile of CBD delivered orally and *i.p.* parallels the PK profile of THC in both humans and rodents, but co-administration of CBD with THC and other select medications impacts their respective PK profiles.¹⁶

CBD modulates the activities of multiple proteins

CBD exhibits an even broader poly-pharmacology profile than THC, with only few common targets. The discussion below starts with those targets validated by genetic deletion or antagonism *in vivo* and ends with those that were reported using heterologous expression systems, systematically emphasizing the concentrations of CBD required to modulate these targets in the context of the concentrations it reaches in brain.

CBD modulates the activity of several GPCRs. Early studies established that CBD binds poorly to the orthosteric site of CB₁R, and however, it antagonizes CB₁R signaling (Figure 8).^{31,232} Studies of CB₁R tertiary structure and signaling suggest that CBD is a negative allosteric modulator of CB₁R, most likely by binding to its N termini, one of the longest among class A GPCRs.^{55,233} Specifically, the current structure-based model of CB₁R transition between inactive and active state posits that CBD might bind to the N termini of CB₁R, resulting in a change in the 3 dimensional structure of the orthosteric binding site and in THC's and 2-AG's potencies.²³³ In line with this model, CBD (0.1–1 μ M) reduces the efficacies and potencies of both 2-AG and THC by activating CB₁R signaling in neurons in cell culture, and inhibits eCB-mediated synaptic plasticity without influencing excitatory transmission.^{234–236} Although these results suggest that CBD inhibits CB₁R signaling by directly interacting with an allosteric binding site on this target, a likely mechanism for some of CBD's therapeutic properties and diminishing the impairing consequences linked to CB₁R activation, direct demonstration of CBD binding to CB₁R is still needed (see below).

CBD (1–5 μ M) is a partial agonist at GPR55, a GPCR involved in the regulation of cell division and neuronal pathfinding during development, and regulates neurotransmission in the adult brain (Figure 8).^{237,238} By partially activating GPR55 and antagonizing its endogenous activation by lysophosphatidyl inositol, CBD most likely increases GABAergic inhibitory neurotransmission in the hippocampus and dampens hippocampal overexcitation

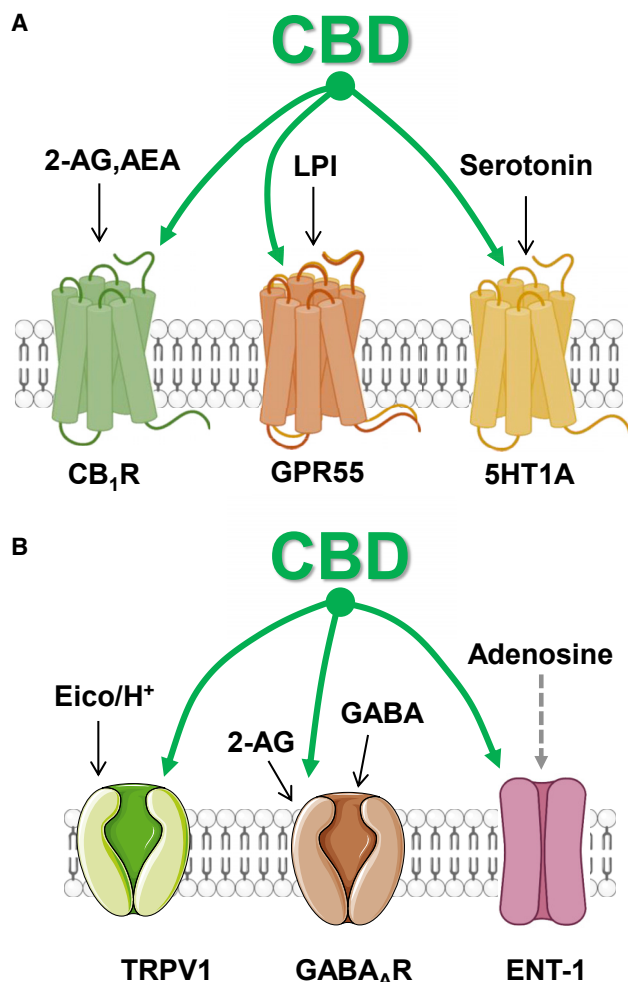


Figure 8. Molecular targets modulated by CBD

(A) CBD is a negative allosteric modulator of CB₁R that is endogenously activated by 2-AG and AEA, an antagonist of GPR55 that is endogenously activated by lysophosphatidyl inositol (LPI) and activate 5HT_{1A} that is endogenously activated by serotonin.

(B) CBD is an agonist at TRPV1 that is activated by eicosanoids (Eico) and changes in H⁺, a positive allosteric modulator of GABA_AR that is activated by GABA and endogenously allosterically modulated by 2-AG, and a blocker of ENT-1 transporters that carry adenosine.

in epilepsy^{63,239–241} (Figure 9). *In vitro* experiments suggest that CBD antagonizes 5-HT_{1A} receptors; but only when reaching 100 μM, a surprising result considering that some of CBD's bioactivity is mediated through this GPCR *in vivo* (see below).²⁴²

Cell culture evidence suggests that additional GPCRs are modulated by CBD. Computational and cell culture functional validation suggest that CBD (10 μM) antagonizes orexin 1 receptor of type 1 (OX1R), a key regulator in arousal and the sleep/wake cycle, as well as motivation and reward processes.²⁴³ CBD acts as a negative allosteric modulate at mu- and delta-opioid receptors, although this effect occurs at 30–100 μM.²⁴⁴ Considering that CBD reaches at maximum of 3 μM in the brain following 120 mg/kg i.p. injections, the involvement of these targets in mediating CBD's bioactivity still requires *in vivo* validation.²²⁸

CBD modulates the activity of ion channels and protein transporters (Figure 8). Of relevance to the sedative response to high-dose CBD, we now have a good understanding of the molecular mechanism by which CBD increases GABA_AR currents. Several endogenous mechanisms increase tonic GABA_AR currents through allosteric modulation, including neurosteroids and 2-AG, and thus contribute to enhancing inhibitory neurotransmission.^{51,245} Cell culture evidence shows that 2-AG (0.3–30 μM) and CBD (3–30 μM) allosterically increase GABA_AR currents by 4-fold, although a mechanism that does not involve the benzodiazepine and neurosteroid binding sites.^{230,246–248} Although the resolution of the GABA_AR crystal structure outlined an allosteric site in hydrophobic tails that is distinct from the site engaged by neurosteroids and possibly cholesterol, whether CBD acts by binding to this site has not been directly tested.^{249,250} CBD potentiation of tonic GABA_AR currents could also be responsible for dampening hyperexcitation and seizures and may cause sedation at high concentrations.²⁵¹ These studies suggest that small molecules that directly target allosteric sites on GABA_AR engaged by 2-AG and possibly CBD, or by inhibiting 2-AG hydrolysis, (e.g., with ABHD6 inhibitors) represent promising therapeutic approaches to reduce enhanced excitatory transmission and treat select neurological diseases, including epilepsy.⁵¹

CBD, acting through transient receptor potential cation channel subfamily V member 1 (TRPV1), is anxiolytic in rats and analgesic in a rat model of acute inflammation (Figure 8).^{252,253} Cell culture studies suggest that CBD activates and desensitizes TRPV1.²⁵⁴ Computational approaches suggest that upregulation of TRPV channels might unmask CBD bioactivity at TRPV2, TRPV3, TRPV4, TRPA1, and TRPM8.^{255,256} Although the molecular mechanism by which CBD might regulate TRP channels remains unknown, a better understanding of its TRPV1-mediated bioactivity *in vivo* represents a growing area of study.

CBD is a competitive inhibitor of the equilibrative nucleoside transporter-1 (IC₅₀ < 200 nM), a primary inactivation mechanism for the signaling function of extracellular adenosine that is expressed by neurons and glia.^{257–259} Thus, inhibition of this transporter by CBD blocks adenosine reuptake by neurons and glia increases local adenosine levels and its ensuing activity at its receptors, A2a. Through this mechanism of action, CBD influences several behaviors, including reducing anxiety (see below). Cell culture studies suggest that CBD (3–10 μM) inhibits currents mediated by the voltage-gated sodium channel Nav1.4 and the voltage-dependent anion channel 1 in mitochondria.^{260–262}

Thus, CBD modulates the activity of several GPCRs, ion channels, and transporters at concentrations that are within the range of the concentrations reached in the brain when treated *in vivo*. The involvement of most of these targets in CBD's bioactivity *in vivo* remain to be demonstrated but should guide the development of novel therapeutics.

CBD BIOACTIVITY IN HUMANS AND RODENTS AND BACK

CBD has been considered non-psychotropic because, in humans, oral consumption of CBD up to 300 mg and i.v. injection of CBD up to 30 mg are both felt as being inactive.^{263,264}

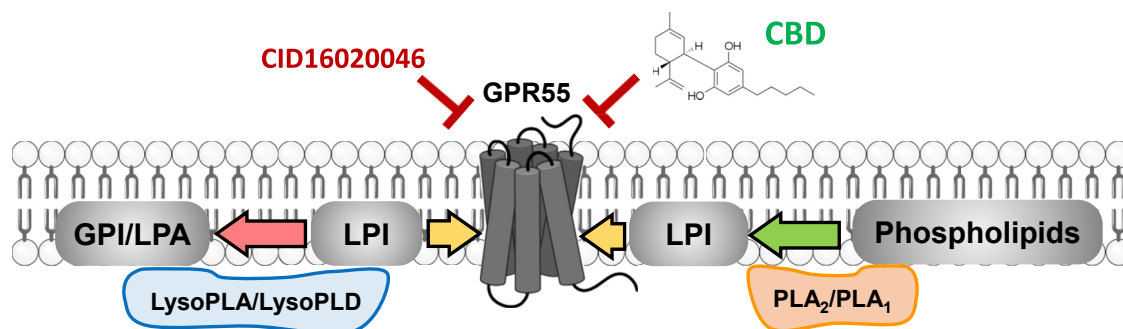


Figure 9. LPI-GPR55 endogenous signaling

LPI is produced by PLA₁/PLA₂, activates GPR55, and is inactivated by lysophospholipase A (LysoPLA) and lysophospholipase D (LysoPLD). CBD antagonizes GPR55, as well as the antagonist CID160200465.

Although recent human studies confirmed that even high doses of oral CBD do not cause THC-like or *Cannabis*-like effects,²⁶⁵ additional studies showed that low- and high-dose CBD does influence human behaviors, and thus, CBD should be considered psychoactive. For example, inhaled CBD (12.5 mg) enhances verbal episodic memory in healthy young participants.²⁶⁶

CBD also influences rodent behavior when administered as a single agent and at low dose. CBD (10 mg/kg i.p.) impairs memory reconsolidation in rats through a CB₁R-dependent mechanism and possibly by reducing Zif268/Egr1 expression, a transcription factor proxy for synaptic plasticity related to reconsolidation.^{119,267} CBD (20 mg/kg i.p.) affects motor behavior in rats as indicated by reduction in vertical activity (i.e., rearing which is a context-sensitive behavior) through a 5-HT_{1A}-dependent mechanism.^{268,269} Acute infusions of CBD (100 ng over 5 min) into the PFC in rats impairs attentional set-shifting and spatial working memory, without interfering with anxiety or sociability behaviors.¹⁶² Mechanistically, single and repeated exposure to CBD (5–30 mg/kg, i.p.) differently modulate BDNF expression and signaling in rat cortex and striatum.²⁷⁰ Thus, rodent studies emphasize that CBD influences key brain functions, including motor behavior and memory reconsolidation, and emphasizes its psychoactivity at low dose.

CBD therapeutic indications

The successful clinical trial demonstrating CBD's antiseizure properties in young adults with refractory seizures represents yet another landmark in the study of its bioactivity. Specifically, a multinational, randomized, double-blind trial of adjunctive CBD (300 mg/kg, oral) versus placebo showed significant reduction in seizures in adolescent patients with Dravet syndrome and Lennox-Gastaut syndrome and caused only mild side effects.^{240,271} The early finding that CBD metabolism competes with clobazam metabolism at CYP3A4 led to speculation that the antiseizure efficacy of CBD may simply reflect CBD augmenting clobazam exposure and intrinsic activity; however, human studies indicated that CBD reduces seizure frequency both with and without concomitant clobazam and that these two antiepileptic treatments appear to produce additive benefit.^{231,272} Thus, combination therapy treatments using CBD and low-dose clobazam represents a promising approach to

control seizures while reducing the side effects linked to clobazam.^{231,272–275}

Rodent studies provided an initial mechanistic understanding of the antiseizure qualities of CBD. CBD (30–100 mg/kg) reduces epileptiform and seizure responses as measured by electrophysiological recordings of rodents' hippocampal slices and dampens seizures in rodent model systems of chemically induced seizures as well as in *Scn1a*^{+/-} mice, a genetic mouse model of Dravet syndrome.^{239,276–278} Furthermore, CBD potently inhibits CYP3A4 mediated metabolism of clobazam in *Scn1a*^{+/-} mice. CBD-clobazam combination treatment achieves greater antiseizure responses, but only when an antiseizure dose of CBD is used.²³⁰ Thus, in this preclinical mouse model of Dravet syndrome, CBD and clobazam produce additive effects, a dual mechanism likely to involve competition for metabolism and independent potentiation of GABA_AR, results that are guiding the design of future human clinical trials.²³⁰ Of note, although CBD use at high concentrations produced notable side effects in only select patients in the clinical trial setting, the effects of its chronic use in combination with other medications, and their mechanistic interactions, remain largely unknown.

Two additional therapeutic opportunities with CBD may be in anxiety and sleep quality.²⁷⁹ Early evidence in double-blind randomized design trials suggested that CBD (400–600 mg, oral, acute) decreases anxiety as measure by survey and functional neuroimaging (i.e., regional cerebral blood flow at rest) in naive patients with generalized social anxiety disorder.^{280,281} However, several trials challenged this result, including a randomized double-blind, placebo-controlled trial that tested acute oral CBD (600 mg) and measured emotional processing and neuroimaging.²⁸² Remarkably, lower dose CBD might represent a more promising approach as suggested by a single patient case reports and small retrospective study with CBD (18–25 mg, oral, chronic) reporting reduced sustained anxiety and improved sleep.^{283,284} Based on this premise, CBD treatment might help with anxiety and sleep, but significant optimization besides dose adjustment will be required. Preclinical evidence in rats might provide important clues as direct injection of CBD into the PFC induces antidepressant-like effect, and injection into the periaqueductal gray blocks panic-like response by activating 5-HT_{1A} receptors.^{269,285–287}

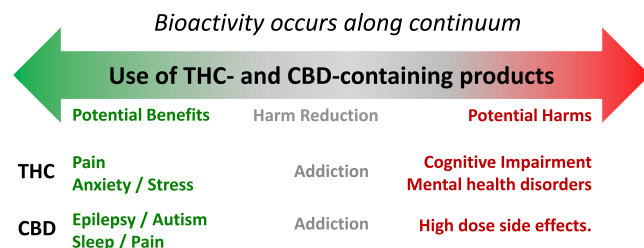


Figure 10. THC and CBD bioactivity occurs along continuum

THC exhibits promising therapeutic response for the treatment of chronic pain (green), potential harm-reduction properties in the context of addiction (gray), and triggers impairing effects and enhances the incidence of mental health disorders (red). CBD exhibits promising therapeutic response for the treatment of epileptic seizures, autism, sleep quality, and chronic pain, potential harm-reduction properties in the context of addiction, and triggers side effects when used at high doses.

CBD appears to have analgesic properties, at least when used at high dose. In humans, CBD (300 mg/oral/daily) prevents acute and transient chemotherapy-induced peripheral neuropathy, but CBD (30 mg/kg) does not affect pain linked to irritable bowel syndrome.^{288,289} In line with these human results, acute CBD in rodents significantly attenuates pain-associated behaviors in neuropathic pain models but yields mixed results in inflammatory pain models.¹⁸³ CBD (0.3–30 mg/kg, i.p.) effectively reverses mechanical and thermal allodynia, hyperalgesia, and anxious behaviors in a rat neuropathic pain model, a response that involves CB₁R and TRPV1.²⁹⁰ Furthermore, the GRP55 antagonist, CID16020046, exhibits promising antinociceptive properties in a rat formalin test, suggesting the involvement of a CBD-GPR55 molecular interaction.^{63,291} Thus, CBD has evident analgesic properties that involve multiple protein targets (Figure 8).

What are the next most promising therapeutic indications for CBD? Monitoring practice-based evidence in patients can provide clues for new therapeutic approaches. The first striking example is autism spectrum disorder, which is characterized by persistent deficits in social communication; restricted and repetitive patterns of behavior, interests, or activities; and often intellectual disabilities.^{292,293} CBD (4–7 mg/kg/day for 6–9 months) is associated with some level of improvement in attention deficit, hyperactivity, communication deficits, social interaction deficits, and cognitive deficits, as well as sleep, a prevalent comorbidity of this syndrome.²⁹⁴ CBD (600 mg/oral) alters regional low-frequency activity and functional connectivity in the brain of adults diagnosed with autism spectrum disorder as measured by fMRI signals.²⁹⁵ A telling example provided by preclinical studies is that CBD bioactivity is also often biphasic, producing one set of behavioral changes at low doses and exhibiting distinctly different bioactivity at high doses. Specifically, CBD (5–20 mg/kg) improves autistic-like social interaction deficits in adult *Scn1a*^{+/-} mice, a preclinical model of Dravet syndrome, and only at a higher dose (100 mg/kg) reduces seizures in adolescent *Scn1a*^{+/-} mice.^{207,239} Furthermore, CBD (5 mg/kg) attenuates aggressive behavior induced by social isolation in mice through CB₁R and 5-HT_{1A} receptor-dependent mechanisms.²⁹⁶

The second example of CBD's potential is for the treatment of drug abuse, especially opioid addiction.²⁹⁷ Human trial

testing of CBD showed that it reduces cue-induced craving and anxiety in drug-abstinent individuals with heroin use disorder.²⁹⁸ Similarly, CBD shows promising therapeutic efficacy in several rodent models of addiction. CBD (5–20 mg/kg) reduces heroin-triggered anxiety-like behavior, motor activity, and somatic signs in mice.²⁹⁹ Mechanistically, this treatment normalizes the altered expression profile of the mu-opioid receptor, proopiomelanocortin, and CB₁R expression in the VTA of mice exposed to spontaneous heroin withdrawal.²⁹⁹ Thus, CBD has promising adjunctive management properties for opioid withdrawal.³⁰⁰ CBD (20 mg/kg) (1) attenuated the rewarding effects of cocaine in rats via CB₂R, 5-HT_{1A}, and TRPV1 receptor mechanisms, (2) decreased motivation for cocaine in a behavioral economics paradigm in mice (i.e., reduced cocaine intake when administered during the acquisition phase), and (3) normalized the expression of GluR2 and pERK1/2 in amygdala.^{301,302} Mechanistically, CB₁R antagonism reverses the effects of CBD on cue- and stress-induced reinstatement of cocaine-seeking behavior in mice, suggesting that CBD acts by allosterically reducing CB₁R signaling and its role in the control of psychostimulant triggered hyperlocomotion.^{98,303} Accordingly, CBD (10 µg/5 µL) infused in the lateral ventricle during the drug conditioning phase impairs the rewarding effects of methamphetamine.³⁰⁴ Finally, rodent studies suggest that CBD might also help with alcohol addiction. For example, CBD (10 mg/kg, acute) attenuates ethanol-induced place preference and reduces aggressivity in group-housed rats.³⁰⁵ Together, this growing preclinical evidence suggests that CBD could potentially be added to the standard detoxification regimen and mitigate acute or protracted withdrawal-related symptoms for several drugs of abuse, including opioids, psychostimulants, and alcohol. Clearly, the therapeutic potential of CBD is only starting to be revealed and preclinical studies are poised to help identify new medical indications.

Is CBD use associated with side effects?

It is noteworthy that CBD does not produce overt impairment as a single agent and thus exhibits a broader therapeutic index than THC (even at relatively high doses). For example, the recent testing of CBD's therapeutic efficacy in multiple human clinical trials reported only mild and transient side effects in some patients (e.g., somnolence, decreased appetite, diarrhea, and fatigue).²⁶⁵ Inhaled CBD (400 mg) followed by a one-week washout period increases the duration and intensity of measures of mismatch negativity amplitude, an auditory event-related potential that occurs when a sequence of repetitive sounds is interrupted by an occasional sound that differs in frequency or duration.³⁰⁶ Furthermore, an observational study on the impact of long-term consumption of oral CBD (40–60 mg/day for 30 days) suggested no increased prevalence of liver toxicity in these self-medicating patients.³⁰⁷ Thus, these studies indicate that a handful of reversible side effects may occur in some individuals who take high doses of CBD (e.g., 400 mg/day for several weeks) and that using 10-fold lower doses (e.g., 40 mg/day for several weeks) may be safe while still significantly influencing behavior.

Interactions between CBD and THC bioactivities

As early as the 1970s, evidence suggested that CBD (40 mg, oral, acute) might interact with THC (20 mg, oral, acute) in humans. For example, CBD delays the onset and prolongs the psychotropic effects of THC.^{308,309} We now know that different doses and routes of administration of CBD have different effects on THC's bioactivity. In humans, inhaled CBD (400 mg) attenuates the duration and intensity of THC (12 mg)-induced changes in mismatch negativity amplitude, the measure of auditory sensory perception.³⁰⁶ However, inhaled CBD (125 mg) does not curb THC (125 mg)-induced impairment of driving and cognition as measured by simulated driving and cognitive performance tests, and in some circumstances, CBD may even exacerbate THC-induced impairment.³¹⁰ Furthermore, inhaled CBD (30 mg) does not protect against the acute adverse effects of *Cannabis* as measured by delayed verbal recall, psychotic symptoms, and subjective measures.³¹¹ Oral CBD (600 mg) attenuates both THC (1.5 mg i.v.)-elicited paranoid symptoms and memory impairments.

The ability of CBD to dampen specific THC's bioactivities in rodents is well documented and depends on the dose administered. For example, in rats, CBD (3 mg/kg i.p.) mitigates the negative anxiogenic effects experienced from THC (1 mg/kg i.p.), a response that correlates with increase dopamine levels in the PFC.³¹² CBD (3 mg/kg i.p.) prevents the THC (3 mg/kg i.p.)-induced downregulation of BDNF function in the hippocampus.³¹³ However, CBD fails to reverse hypothermia or locomotor suppression induced by THC (30 mg/kg i.p.) in rats.³¹⁴

Can CBD help mitigate the detrimental effects of THC in the developing brain? Multiple preclinical studies suggest yes. In adolescent mice treated for 15 days (PND 28–48) with daily i.p. injections of THC (3 mg/kg), CBD (3 mg/kg) reduced the impact of THC.¹⁷ Specifically, THC triggers immediate and long-term impairments in working memory measured by the novel object recognition task, increases adulthood anxiety measured on the elevated plus maze, and increases repetitive and compulsive-like behaviors measured with the Nestlet shredding task and marble burying.¹⁷ All of these THC-induced behavioral abnormalities were dampened by the co-administration of CBD, whereas CBD as a single agent did not generate behavioral outcomes.¹⁷ Treatment of adolescent female rats (PND 35–45, 15 days, twice daily) with increasing doses of THC (2.5, 5, and 10 mg/kg) impairs emotional behaviors in adulthood as measured by the swim test, sucrose intake, palatable food intake, and elevated plus maze.^{299,315} Accordingly, CBD mitigates select long-term behavioral alterations and molecular changes in the PFC.^{299,315} Intra-PFC injection of CBD reverses the cognitive impairments induced by acute glutamatergic antagonism within the PFC and blocks the anxiogenic properties of THC.³¹⁵ These results suggest that CBD mitigates some of the long-term behavioral alterations induced by adolescent THC exposure and diminishes the long-term changes in PFC molecular components.

In summary, multiple, independent, preclinical studies have shown that the effects of THC use during adolescence and adulthood are lessened by CBD. The molecular mechanism by which CBD decreases THC's effect on the brain remains to be established *in vivo* but likely involves the negative allosteric modulation

of CB₁R. Further studies are urgently needed to help develop therapeutics for such conditions as CUD and THC-triggered psychosis and schizophrenia.

OUTLOOK

This review raises questions that point to future research directions. First, we need more, large, placebo-controlled studies to solidify the therapeutic properties of both THC and CBD when using several ratios, including their PK and PD mechanistic interactions, testing their therapeutic potential in indications suggested by preclinical studies. This commitment will result in the development of multiple new transformative therapeutic options for the treatment of devastating diseases that remain without standard care options. Second, we need better definitions of “psychotropic,” “non-psychotropic,” and “impairing.” In particular, we need to better understand to what extent individuals can self-titrate. For example, individuals who smoke *Cannabis* containing THC, *Cannabis* containing THC-CBD, or vaping THC concentrates self-titrate to reach comparable intoxicating levels.³¹⁶ Remarkably, self-titration of THC is also measured in rodents.^{21,22} Accordingly, it is critical to develop reliable measures of acute THC and CBD bioactivity in preclinical models and humans when administered using common routes (inhaled and oral). Considering that 11-OH- Δ^9 -THC exhibits comparable activity at CB₁R and accumulation in the brain to THC, could this THC metabolite significantly contribute to THC's bioactivity profile when administered orally? What is the role of P450 enzymes expressed in the brain that metabolize THC and CBD?³¹⁷ How does the bioactivity of artificial cannabinoid agonists compare with THC? For example, what is the bioactivity of JWH-018, a high-affinity and full agonist at CB₁R, often infused in illegal products such as *Spice* and *K2*, that can be abused?^{177,318}

It remains possible that chronic use of high-dose CBD may produce side effects in vulnerable individuals, including during development and when used in combination with other medications. For example, considering that CBD modulates the activity of proteins involved in neuronal pathfinding and maturation, such as CB₁R and GPR55, we still do not know what the impact of high-dose CBD is on brain development? What medication interact pharmacologically with CBD? We have started to outline the pharmacological interactions between THC and other psychoactive drugs, such as opioids, but we still lack research on CBD's pharmacological interactions. For example, considering that CBD allosterically modulates GABA_AR, does co-administration of benzodiazepine and CBD increase concentration difficulties, confusion, sedation, and motor incoordination? To answer these questions, we need to further define the single agent bioactivities of CBD at various doses and administered through distinct routes, determine its selectivity at specific targets, and consider whether CBD tissue exposure reaches concentrations that will significantly engage and modulate these targets *in vivo*.

THC and CBD as single agents do not represent the full bioactive profile of *Cannabis* which contains additional bioactive phyto-CBs and ingredients, such as terpenes.³¹⁹ Thus, another blooming area of research is the bioactivity, mechanism of action, and effects of THC analogs that are produced by *Cannabis*

at low levels and that activate CB₁R similarly to THC, such as Δ^8 -THC and Δ^9 -tetrahydrocannabiphinol. These analogs can be concentrated via extraction procedures.^{320,321} For example, Δ^8 -THC (1–6 mg) triggers comparable but less potent behavioral changes in healthy human adults than Δ^9 -THC at a similar dose.^{73,322} This research is particularly important considering the recent increase in the United States of products that contain Δ^8 -THC instead of Δ^9 -THC.³²³

Conclusions

Research on the bioactivity of THC and CBD started in the 1960s, initially focusing on THC and more recently rediscovering CBD. We now have a foundational understanding of their distinct molecular mechanisms of action and how they modify neuronal and glial cell functions in the brain over an individual's lifetime. Key results, gathered in well-controlled human studies and validated in preclinical model systems, have set the stage for the optimization and development of novel, phyto-CB-based therapeutics for a broad array of devastating neurological and psychiatric diseases. This review summarizes impairing consequences and side effects alongside potential therapeutics, emphasizing the importance of establishing THC and CBD dosages and regimens based on bioactivity (Figure 10). Considering the wide differences and diversity in the molecular targets engaged by THC and CBD, phyto-CB-based therapeutics will require optimization for each medical indication, from sleep disorders, anxiety, and drug abuse to refractory childhood epilepsy and autism.

Recent development of molecular tools and experimental approaches, validated in rodents, create an opportunity to provide practical and translatable results for humans. Molecular imaging technologies now enable the visualization of targets engaged by phyto-CBs, including positron emission tomography (PET) ligands that target and image CB₁R in vivo and can be leveraged to study CB₁R pharmacological interaction with other psychoactive drugs, such as nicotine and alcohol.^{324,325} Biosensors that selectively measure changes in THC, CBD, and eCB levels in tissue are paving the way to establish the precise real-time changes of these agents in different tissues and how that relates to their bioactivities.^{326–328}

Much of the *Cannabis* and cannabinoid research over the past 50 years was done in reverse: listening to users to understand bioactivity and to patients to recognize therapeutic benefits before carrying out the needed trials. A perfect illustration of such a research trajectory was the development of CBD-containing therapeutics for the treatment of epilepsies: patients and their families spurred the study and optimization of the anti-seizure properties of CBD, and this novel therapeutic approach was eventually proven in preclinical studies and human trials. The current thriving and innovative field of *Cannabis* and cannabinoid research is reversing this trajectory. We are poised to optimize cannabinoids, develop multiple lines of transformative medications, and guide their safe use in the growing legal market of *Cannabis*-based products.

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REFERENCES

1. Carlini, B.H., and Schauer, G.L. (2022). Cannabis-only use in the USA: prevalence, demographics, use patterns, and health indicators. *J. Cannabis Res.* 4, 1–8.
2. National Academies of Sciences, Engineering, and Medicine (2017). The health effects of Cannabis and Cannabinoids: The current state of evidence and recommendations for research. <https://doi.org/10.17226/24625>.
3. Hines, L.A., Freeman, T.P., Gage, S.H., Zammit, S., Hickman, M., Cannon, M., Munafò, M., MacLeod, J., and Heron, J. (2020). Association of high-potency cannabis use with mental health and substance use in adolescence. *JAMA Psychiatry* 77, 1044–1051.
4. Hasin, D.S., Sarvet, A.L., Cerdá, M., Keyes, K.M., Stohl, M., Galea, S., and Wall, M.M. (2017). US adult illicit cannabis use, cannabis use disorder, and medical marijuana laws. *JAMA Psychiatry* 74, 2012–2013.
5. Smart, R., Caulkins, J.P., Kilmer, B., Davenport, S., and Midgette, G. (2017). Variation in cannabis potency and prices in a newly legal market: evidence from 30 million cannabis sales in Washington state. *Addiction* 112, 2167–2177.
6. Freeman, T.P., and Lorenzetti, V. (2020). ‘Standard THC units’: a proposal to standardize dose across all cannabis products and methods of administration. *Addiction* 115, 1207–1216.
7. Melzer, R., McCabe, P.F., and Schilling, S. (2022). Evolution, genetics and biochemistry of plant cannabinoid synthesis: a challenge for biotechnology in the years ahead. *Curr. Opin. Biotechnol.* 75, 102684. <https://doi.org/10.1016/j.copbio.2022.102684>.
8. Campbell, L.G., Dufresne, J., and Sabatino, S.A. (2020). Cannabinoid inheritance relies on complex genetic architecture. *Cannabis Cannabinoid Res.* 5, 105–116.
9. Barrus, D.G., Lefever, T.W., and Wiley, J.L. (2018). Evaluation of reinforcing and aversive effects of voluntary Δ^9 -tetrahydrocannabinol ingestion in rats. *Neuropharmacology* 137, 133–140.
10. Newmeyer, M.N., Swortwood, M.J., Barnes, A.J., Abulseoud, O.A., Scheidweiler, K.B., and Huestis, M.A. (2016). Free and glucuronide whole blood cannabinoids' pharmacokinetics after controlled smoked, vaporized, and oral cannabis administration in frequent and occasional cannabis users: identification of recent cannabis intake. *Clin. Chem.* 62, 1579–1592.
11. Marsot, A., Audebert, C., Attolini, L., Lacarelle, B., Micallef, J., and Blin, O. (2016). Comparison of cannabinoid concentrations in plasma, oral fluid and urine in occasional cannabis smokers after smoking cannabis cigarette. *J. Pharm. Pharm. Sci.* 19, 411–422.
12. Grant, K.S., Petroff, R., Isoherranen, N., Stella, N., and Burbacher, T.M. (2018). Cannabis use during pregnancy: Pharmacokinetics and effects on child development. *Pharmacol. Ther.* 182, 133–151. <https://doi.org/10.1016/j.pharmthera.2017.08.014>.
13. Huang, H., McIntosh, A.L., Martin, G.G., Dangott, L.J., Kier, A.B., and Schroeder, F. (2018). Structural and functional interaction of δ^9 -tetrahydrocannabinol with liver fatty acid binding protein (FABP1). *Biochemistry* 57, 6027–6042.
14. Dinis-Oliveira, R.J. (2016). Metabolomics of Δ^9 -tetrahydrocannabinol: Implications in toxicity. *Drug Metab. Rev.* 48, 80–87.
15. Parikh, N., Kramer, W.G., Khurana, V., Cognata Smith, C.C., and Vettica-den, S. (2016). Bioavailability study of dronabinol oral solution versus dronabinol capsules in healthy volunteers. *Clin. Pharmacol.* 8, 155–162.

16. Karschner, E.L., Darwin, W.D., Goodwin, R.S., Wright, S., and Huestis, M.A. (2011). Plasma cannabinoid pharmacokinetics following controlled oral Δ^9 -tetrahydrocannabinol and oromucosal cannabis extract administration. *Clin. Chem.* 57, 66–75.
17. Murphy, M., Mills, S., Winstone, J., Leishman, E., Wager-Miller, J., Bradshaw, H., and Mackie, K. (2017). Chronic adolescent Δ^9 -tetrahydrocannabinol treatment of male mice leads to long-term cognitive and behavioral dysfunction, which are prevented by concurrent cannabidiol treatment. *Cannabis Cannabinoid Res.* 2, 235–246.
18. Dow-Edwards, D., and Zhao, N. (2008). Oral THC produces minimal behavioral alterations in preadolescent rats. *Neurotoxicol. Teratol.* 30, 385–389.
19. Torrens, A., Vozella, V., Huff, H., McNeil, B., Ahmed, F., Ghidini, A., Mahler, S.V., Huestis, M.A., Das, A., and Piomelli, D. (2020). Comparative pharmacokinetics of Δ^9 -tetrahydrocannabinol in adolescent and adult male mice. *J. Pharmacol. Exp. Ther.* 374, 151–160.
20. Manwell, L.A., Charchoglyan, A., Brewer, D., Matthews, B.A., Heipel, H., and Mallet, P.E. (2014). A vaporized Δ^9 -tetrahydrocannabinol (Δ^9 -THC) delivery system part I: Development and validation of a pulmonary cannabinoid route of exposure for experimental pharmacology studies in rodents. *J. Pharmacol. Toxicol. Methods* 70, 120–127.
21. Kruse, L.C., Cao, J.K., Viray, K., Stella, N., and Clark, J.J. (2019). Voluntary oral consumption of Δ^9 -tetrahydrocannabinol by adolescent rats impairs reward-predictive cue behaviors in adulthood. *Neuropsychopharmacology* 44, 1406–1414.
22. Abraham, A.D., Leung, E.J.Y., Wong, B.A., Rivera, Z.M.G., Kruse, L.C., Clark, J.J., and Land, B.B. (2020). Orally consumed cannabinoids provide long-lasting relief of allodynia in a mouse model of chronic neuropathic pain. *Neuropsychopharmacology* 45, 1105–1114.
23. Smoker, M.P., Mackie, K., Lapish, C.C., and Boehm, S.L. (2019). Self-administration of edible Δ^9 -tetrahydrocannabinol and associated behavioral effects in mice. *Drug Alcohol Depend.* 199, 106–115.
24. Freels, T.G., Baxter-Potter, L.N., Lugo, J.M., Glodosky, N.C., Wright, H.R., Baglot, S.L., Petrie, G.N., Yu, Z., Clowers, B.H., Cuttler, C., et al. (2020). Vaporized cannabis extracts have reinforcing properties and support conditioned drug-seeking behavior in rats. *J. Neurosci.* 40, 1897–1908.
25. Taffe, M.A., Creehan, K.M., Vandewater, S.A., Kerr, T.M., and Cole, M. (2021). Effects of Δ^9 -tetrahydrocannabinol (THC) vapor inhalation in Sprague-Dawley and Wistar rats. *Exp. Clin. Psychopharmacol.* 29, 1–13.
26. Ruiz, C.M., Torrens, A., Lallai, V., Castillo, E., Manca, L., Martinez, M.X., Justeson, D.N., Fowler, C.D., Piomelli, D., and Mahler, S.V. (2021). Pharmacokinetic and pharmacodynamic properties of aerosolized (“vaped”) THC in adolescent male and female rats. *Psychopharmacology* 238, 3595–3605.
27. Narimatsu, S., Watanabe, K., Yamamoto, I., and Yoshimura, H. (1991). Sex difference in the oxidative metabolism of Δ^9 -tetrahydrocannabinol in the rat. *Biochem. Pharmacol.* 41, 1187–1194.
28. Spiro, A.S., Wong, A., Boucher, A.A., and Arnold, J.C. (2012). Enhanced brain disposition and effects of Δ^9 -tetrahydrocannabinol in P-glycoprotein and breast cancer resistance protein knockout mice. *PLoS ONE* 7, e35937.
29. Bonhomme-Faivre, L., Benyamina, A., Reynaud, M., Farinotti, R., and Abbada, C. (2008). PRECLINICAL STUDY: disposition of Δ^9 tetrahydrocannabinol in CF1 mice deficient in mdr1a P-glycoprotein. *Addict. Biol.* 13, 295–300.
30. Matheson, J., Zhang, Y.J., Brands, B., Wickens, C.M., Tiwari, A.K., Zai, C.C., Kennedy, J.L., and Le Foll, B. (2022). Association between ABCB1 rs2235048 Polymorphism and THC Pharmacokinetics and Subjective Effects following Smoked cannabis in Young Adults. *Brain Sci.* 12, 1189.
31. Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., and Bonner, T.I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346, 561–564.
32. Huestis, M.A., Gorelick, D.A., Heishman, S.J., Preston, K.L., Nelson, R.A., Moolchan, E.T., and Frank, R.A. (2001). Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. *Arch. Gen. Psychiatry* 58, 322–328.
33. Lutz, B. (2020). Neurobiology of cannabinoid receptor signaling. *Dialogues. Clin. Neurosci.* 22, 207–222.
34. Manning, J.J., Green, H.M., Glass, M., and Finlay, D.B. (2021). Pharmacological selection of cannabinoid receptor effectors: signalling, allosteric modulation and bias. *Neuropharmacology* 193, 108611. <https://doi.org/10.1016/j.neuropharm.2021.108611>.
35. Leo, L.M., and Abood, M.E. (2021). CB1 cannabinoid receptor signaling and biased signaling. *Molecules* 26, 5413.
36. Horváth, V.B., Soltész-Katona, E., Wisniewski, É., Rajki, A., Halász, E., Enyedi, B., Hunyady, L., Tóth, A.D., and Szanda, G. (2021). Optimization of the heterologous expression of the cannabinoid Type-1 (CB1) receptor. *Front. Endocrinol.* 12, 740913.
37. Costas-Insua, C., Moreno, E., Maroto, I.B., Ruiz-Calvo, A., Bajo-Grañeras, R., Martín-Gutiérrez, D., Díez-Alarcia, R., Vilaró, M.T., Cortés, R., García-Font, N., et al. (2021). Identification of BiP as a CB1 receptor-interacting protein that fine-tunes cannabinoid signaling in the mouse brain. *J. Neurosci.* 41, 7924–7941.
38. Smith, T.H., Blume, L.C., Straiker, A., Cox, J.O., David, B.G., McVoy, J.R., Sayers, K.W., Poklis, J.L., Abdullah, R.A., Egertová, M., et al. (2015). Cannabinoid receptor-interacting protein 1a modulates CB1 receptor signaling and regulation. *Mol. Pharmacol.* 87, 747–765.
39. Busquets-García, A., Bains, J., and Marsicano, G. (2018). CB1 Receptor Signaling in the Brain: Extracting Specificity from Ubiquity. *Neuropsychopharmacology* 43, 4–20.
40. Stella, N. (2010). Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia* 58, 1017–1030. <https://doi.org/10.1002/glia.20983>.
41. Eraso-Pichot, A., Pouvreau, S., Olivera-Pinto, A., Gomez-Sotres, P., Skupio, U., and Marsicano, G. (2023). Endocannabinoid signaling in astrocytes. *Glia* 71, 44–59.
42. Molina-Holgado, E., Esteban, P.F., Arevalo-Martin, Á., Moreno-Luna, R., Molina-Holgado, F., and García-Ovejero, D. (2023). Endocannabinoid signaling in oligodendroglia. *Glia* 71, 91–102.
43. Vendel, E., and de Lange, E.C. (2014). Functions of the CB1 and CB2 receptors in neuroprotection at the level of the blood–brain barrier. *Neuro-Molecular Med.* 16, 620–642.
44. Chung, Y.C., Shin, W.H., Baek, J.Y., Cho, E.J., Baik, H.H., Kim, S.R., Won, S.Y., and Jin, B.K. (2016). CB2 receptor activation prevents glial-derived neurotoxic mediator production, BBB leakage and peripheral immune cell infiltration and rescues dopamine neurons in the MPTP model of Parkinson’s disease. *Exp. Mol. Med.* 48, e205.
45. Maresz, K., Carrier, E.J., Ponomarev, E.D., Hillard, C.J., and Dittel, B.N. (2005). Modulation of the cannabinoid CB receptor in microglial cells in response to inflammatory stimuli. *J. Neurochem.* 95, 437–445.
46. Van Sickle, M.D., Duncan, M., Kingsley, P.J., Mouihate, A., Urbani, P., Mackie, K., Stella, N., Makriyannis, A., Piomelli, D., Davison, J.S., et al. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 310, 329–332. <https://doi.org/10.1126/science.1115740>.
47. Zoratti, C., Kipmen-Korgun, D., Osibow, K., Malli, R., and Graier, W.F. (2003). Anandamide initiates Ca²⁺ signaling via CB2 receptor linked to phospholipase C in calf pulmonary endothelial cells. *Br. J. Pharmacol.* 140, 1351–1362.
48. Lu, H.C., and Mackie, K. (2021). Review of the endocannabinoid system. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* 6, 607–615.
49. Araque, A., Castillo, P.E., Manzoni, O.J., and Tonini, R. (2017). Synaptic functions of endocannabinoid signaling in health and disease. *Neuropharmacology* 124, 13–24.

50. Janssen, F.J., and van der Stelt, M. (2016). Inhibitors of diacylglycerol lipases in neurodegenerative and metabolic disorders. *Bioorg. Med. Chem. Lett.* 26, 3831–3837.
51. Cao, J.K., Kaplan, J., and Stella, N. (2019). ABHD6: its place in endocannabinoid signaling and beyond. *Trends Pharmacol. Sci.* 40, 267–277.
52. van Egmond, N., Straub, V.M., and van der Stelt, M. (2021). Targeting endocannabinoid signaling: FAAH and MAG lipase inhibitors. *Annu. Rev. Pharmacol. Toxicol.* 61, 441–463.
53. Bernal-Chico, A., Tepavcevic, V., Manterola, A., Utrilla, C., Matute, C., and Mato, S. (2023). Endocannabinoid signaling in brain diseases: emerging relevance of glial cells. *Glia* 71, 103–126.
54. Kelley, B.G., and Thayer, S.A. (2004). Delta 9-tetrahydrocannabinol antagonizes endocannabinoid modulation of synaptic transmission between hippocampal neurons in culture. *Neuropharmacology* 46, 709–715.
55. Shao, Z., Yan, W., Chapman, K., Ramesh, K., Ferrell, A.J., Yin, J., Wang, X., Xu, Q., and Rosenbaum, D.M. (2019). Structure of an allosteric modulator bound to the CB1 cannabinoid receptor. *Nat. Chem. Biol.* 15, 1199–1205.
56. Hua, T., Li, X., Wu, L., Iliopoulos-Tsoutsouvas, C., Wang, Y., Wu, M., Shen, L., Brust, C.A., Nikas, S.P., Song, F., et al. (2020). Activation and signaling mechanism revealed by cannabinoid receptor-Gi complex structures. *Cell* 180, 655–665.e18.
57. Vallée, M., Vitiello, S., Bellocchio, L., Hébert-Chatelain, E., Monlezun, S., Martin-Garcia, E., Kasanetz, F., Baillie, G.L., Panin, F., Cathala, A., et al. (2014). Pregnenolone can protect the brain from cannabis intoxication. *Science* 343, 94–98.
58. Busquets-Garcia, A., Soria-Gómez, E., Redon, B., Mackenbach, Y., Vallée, M., Chaouloff, F., Varilh, M., Ferreira, G., Piazza, P.V., and Marsicano, G. (2017). Pregnenolone blocks cannabinoid-induced acute psychotic-like states in mice. *Mol. Psychiatry* 22, 1594–1603.
59. Xiong, W., Wu, X., Li, F., Cheng, K., Rice, K.C., Lovinger, D.M., and Zhang, L. (2012). A common molecular basis for exogenous and endogenous cannabinoid potentiation of glycine receptors. *J. Neurosci.* 32, 5200–5208.
60. Yang, Z., Aubrey, K.R., Alroy, I., Harvey, R.J., Vandenberg, R.J., and Lynch, J.W. (2008). Subunit-specific modulation of glycine receptors by cannabinoids and N-arachidonyl-glycine. *Biochem. Pharmacol.* 76, 1014–1023.
61. Xiong, W., Cheng, K., Cui, T., Godlewski, G., Rice, K.C., Xu, Y., and Zhang, L. (2011). Cannabinoid potentiation of glycine receptors contributes to cannabis-induced analgesia. *Nat. Chem. Biol.* 7, 296–303. <https://doi.org/10.1038/nchembio.552>.
62. Lauckner, J.E., Jensen, J.B., Chen, H.Y., Lu, H.C., Hille, B., and Mackie, K. (2008). GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc. Natl. Acad. Sci. USA* 105, 2699–2704. <https://doi.org/10.1073/pnas.0711278105>.
63. Sylantsev, S., Jensen, T.P., Ross, R.A., and Rusakov, D.A. (2013). Cannabinoid- and lysophosphatidylinositol-sensitive receptor GPR55 boosts neurotransmitter release at central synapses. *Proc. Natl. Acad. Sci. USA* 110, 5193–5198.
64. Lingerfelt, M.A., Zhao, P., Sharir, H.P., Hurst, D.P., Reggio, P.H., and Abood, M.E. (2017). Identification of crucial amino acid residues involved in agonist signaling at the GPR55 receptor. *Biochemistry* 56, 473–486.
65. Foster, S.R., Hauser, A.S., Vedel, L., Strachan, R.T., Huang, X.P., Gavin, A.C., Shah, S.D., Nayak, A.P., Haugaard-Kedström, L.M., Penn, R.B., et al. (2019). Discovery of human signaling systems: pairing peptides to G protein-coupled receptors. *Cell* 179, 895–908.e21.
66. Kapur, A., Zhao, P., Sharir, H., Bai, Y., Caron, M.G., Barak, L.S., and Abood, M.E. (2009). Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. *J. Biol. Chem.* 284, 29817–29827. <https://doi.org/10.1074/jbc.M109.050187>.
67. Pietr, M., Kozela, E., Levy, R., Rimmerman, N., Lin, Y.H., Stella, N., Vogel, Z., and Juknat, A. (2009). Differential changes in GPR55 during microglial cell activation. *FEBS Lett.* 583, 2071–2076. <https://doi.org/10.1016/j.febslet.2009.05.028>.
68. Kallendrusch, S., Kremzow, S., Nowicki, M., Grabiec, U., Winkelmann, R., Benz, A., Kraft, R., Bechmann, I., Dehghani, F., and Koch, M. (2013). The G protein-coupled receptor 55 ligand 1- α -lysophosphatidyl-inositol exerts microglia-dependent neuroprotection after excitotoxic lesion. *Glia* 61, 1822–1831.
69. Barann, M., Molderings, G., Brüss, M., Bönsch, H., Urban, B.W., and Göthert, M. (2002). Direct inhibition by cannabinoids of human 5-HT_{3A} receptors: probable involvement of an allosteric modulatory site. *Br. J. Pharmacol.* 137, 589–596.
70. Xiong, W., Hosoi, M., Koo, B.N., and Zhang, L. (2008). Anandamide inhibition of 5-HT_{3A} receptors varies with receptor density and desensitization. *Mol. Pharmacol.* 73, 314–322.
71. Gaoni, Y., and Mechoulam, R. (1964). Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* 86, 1646–1647.
72. Hollister, L.E. (1970). Tetrahydrocannabinol isomers and homologues: contrasted effects of smoking. *Nature* 227, 968–969.
73. Hollister, L.E., and Gillespie, H.K. (1973). Delta-8- and delta-9-tetrahydrocannabinol comparison in man by oral and intravenous administration. *Clin. Pharmacol. Ther.* 14, 353–357.
74. Holtzman, D., Lovell, R.A., Jaffe, J.H., and Freedman, D.X. (1969). 1- Δ^9 -tetrahydrocannabinol: neurochemical and behavioral effects in the mouse. *Science* 163, 1464–1467.
75. Beardsley, P.M., Scimeca, J.A., and Martin, B.R. (1987). Studies on the agonistic activity of delta 9-11-tetrahydrocannabinol in mice, dogs and rhesus monkeys and its interactions with delta 9-tetrahydrocannabinol. *J. Pharmacol. Exp. Ther.* 241, 521–526.
76. Metna-Laurent, M., Mondésir, M., Grel, A., Vallée, M., and Piazza, P.V. (2017). Cannabinoid-induced tetrad in mice. *Curr. Protoc. Neurosci.* 80, 9.59.1–9.59.10.
77. Monory, K., Blaudzun, H., Massa, F., Kaiser, N., Lemberger, T., Schütz, G., Wotjak, C.T., Lutz, B., and Marsicano, G. (2007). Genetic dissection of behavioural and autonomic effects of Delta(9)-tetrahydrocannabinol in mice. *PLoS Biol.* 5, e269. <https://doi.org/10.1371/journal.pbio.0050269>.
78. Häring, M., Kaiser, N., Monory, K., and Lutz, B. (2011). Circuit specific functions of cannabinoid CB1 receptor in the balance of investigatory drive and exploration. *PLoS ONE* 6, e26617.
79. Han, X., He, Y., Bi, G.H., Zhang, H.Y., Song, R., Liu, Q.R., Egan, J.M., Gardner, E.L., Li, J., and Xi, Z.X. (2017). CB1 receptor activation on VgluT2-expressing glutamatergic neurons underlies Δ^9 -tetrahydrocannabinol (Δ^9 -THC)-induced aversive effects in mice. *Sci. Rep.* 7, 12315.
80. Turner, B.D., Smith, N.K., Manz, K.M., Chang, B.T., Delpire, E., Grueter, C.A., and Grueter, B.A. (2021). Cannabinoid type 1 receptors in A2a neurons contribute to cocaine-environment association. *Psychopharmacology* 238, 1121–1131.
81. Lafenêtre, P., Chaouloff, F., and Marsicano, G. (2009). Bidirectional regulation of novelty-induced behavioral inhibition by the endocannabinoid system. *Neuropharmacology* 57, 715–721. <https://doi.org/10.1016/j.neuropharm.2009.07.014>.
82. Bellocchio, L., Lafenêtre, P., Cannich, A., Cota, D., Puente, N., Grandes, P., Chaouloff, F., Piazza, P.V., and Marsicano, G. (2010). Bimodal control of stimulated food intake by the endocannabinoid system. *Nat. Neurosci.* 13, 281–283. <https://doi.org/10.1038/nn.2494>.
83. Soria-Gomez, E., Pagano Zottola, A.C., Mariani, Y., Desprez, T., Barresi, M., Bonilla-Del Rio, I., Muguruza, C., Le Bon-Jego, M., Julio-Kalajzić, F., Flynn, R., et al. (2021). Subcellular specificity of cannabinoid effects in striatonigral circuits. *Neuron* 109, 1513–1526.e11.
84. Kasten, C.R., Zhang, Y., and Boehm, S.L. (2019). Acute cannabinoids produce robust anxiety-like and locomotor effects in mice, but long-term consequences are age- and sex-dependent. *Front. Behav. Neurosci.* 13, 32.

85. Mitchell, V.A., Harley, J., Casey, S.L., Vaughan, A.C., Winters, B.L., and Vaughan, C.W. (2021). Oral efficacy of $\Delta(9)$ -tetrahydrocannabinol and cannabidiol in a mouse neuropathic pain model. *Neuropharmacology* 189, 108529. <https://doi.org/10.1016/j.neuropharm.2021.108529>.
86. Bruijnzeel, A.W., Qi, X., Guzhva, L.V., Wall, S., Deng, J.V., Gold, M.S., Febo, M., and Setlow, B. (2016). Behavioral characterization of the effects of cannabis smoke and anandamide in rats. *PLoS ONE* 11, e0153327.
87. Vann, R.E., Warner, J.A., Bushell, K., Huffman, J.W., Martin, B.R., and Wiley, J.L. (2009). Discriminative stimulus properties of $\Delta 9$ -tetrahydrocannabinol (THC) in C57BL/6J mice. *Eur. J. Pharmacol.* 615, 102–107.
88. Bass, C.E., and Martin, B.R. (2000). Time course for the induction and maintenance of tolerance to $\Delta 9$ -tetrahydrocannabinol in mice. *Drug Alcohol Depend.* 60, 113–119.
89. Morgan, D.J., Davis, B.J., Kearn, C.S., Marcus, D., Cook, A.J., Wager-Miller, J., Straiker, A., Myoga, M.H., Karduck, J., Leishman, E., et al. (2014). Mutation of putative GPCR phosphorylation sites in the cannabinoid receptor 1 (CB1R) confers resistance to cannabinoid tolerance and hypersensitivity to cannabinoids in mice. *J. Neurosci.* 34, 5152–5163.
90. Parks, C., Jones, B.C., Moore, B.M., and Mulligan, M.K. (2020). Sex and strain variation in initial sensitivity and rapid tolerance to $\Delta 9$ -tetrahydrocannabinol. *Cannabis Cannabinoid Res.* 5, 231–245.
91. Missig, G., Mehta, N., Robbins, J.O., Good, C.H., Iliopoulos-Tsoutsouvas, C., Makriyannis, A., Nikas, S.P., Bergman, J., Carlezon, W.A., and Paronis, C.A. (2022). Altered sleep during spontaneous cannabinoid withdrawal in male mice. *Behav. Pharmacol.* 33, 195–205.
92. Kesner, A.J., Mateo, Y., Abrahao, K.P., Ramos-Maciel, S., Pava, M.J., Gracias, A.L., Paulsen, R.T., Carlson, H.B., and Lovinger, D.M. (2021). Sex-dependent changes in murine striatal dopamine release, sleep, and behavior during spontaneous $\Delta 9$ -tetrahydrocannabinol abstinence. <https://doi.org/10.1101/2021.12.01.470765>.
93. Micallef, J., Dupouey, J., Jouve, E., Truillet, R., Lacarelle, B., Taillard, J., Daurat, A., Authié, C., Blin, O., Rascol, O., et al. (2018). Cannabis smoking impairs driving performance on the simulator and real driving: a randomized, double-blind, placebo-controlled, crossover trial. *Fundam. Clin. Pharmacol.* 32, 558–570.
94. Windle, S.B., Eisenberg, M.J., Reynier, P., Cabaussel, J., Thombs, B.D., Grad, R., Ellis, C., Sequeira, C., and Filion, K.B. (2021). Association between legalization of recreational cannabis and fatal motor vehicle collisions in the United States: an ecologic study. *CMAJ Open* 9, E233–E241.
95. Ledent, C., Valverde, O., Cossu, G., Petitot, F., Aubert, J.F., Beslot, F., Böhme, G.A., Imperato, A., Pedrazzini, T., Roques, B.P., et al. (1999). Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* 283, 401–404.
96. Wiley, J.L. (2003). Sex-dependent effects of $\Delta 9$ -tetrahydrocannabinol on locomotor activity in mice. *Neurosci. Lett.* 352, 77–80.
97. Blázquez, C., Chiarlone, A., Sagredo, O., Aguado, T., Pazos, M.R., Resel, E., Palazuelos, J., Julien, B., Salazar, M., Börner, C., et al. (2011). Loss of striatal type 1 cannabinoid receptors is a key pathogenic factor in Huntington's disease. *Brain* 134, 119–136. <https://doi.org/10.1093/brain/awq278>.
98. Bonm, A.V., Elezgarai, I., Gremel, C.M., Viray, K., Bamford, N.S., Palmer, R.D., Grandes, P., Lovinger, D.M., and Stella, N. (2021). Control of exploration, motor coordination and amphetamine sensitization by cannabinoid CB1 receptors expressed in medium spiny neurons. *Eur. J. Neurosci.* 54, 4934–4952.
99. Walker, F.O. (2007). Huntington's disease. *Lancet* 369, 218–228.
100. Wang, W., Dever, D., Lowe, J., Storey, G.P., Bhansali, A., Eck, E.K., Nituțescu, I., Weimer, J., and Bamford, N.S. (2012). Regulation of prefrontal excitatory neurotransmission by dopamine in the nucleus accumbens core. *J. Physiol.* 590, 3743–3769. <https://doi.org/10.1113/jphysiol.2012.235200>.
101. Bamford, N.S., Wightman, R.M., and Sulzer, D. (2018). Dopamine's effects on corticostriatal synapses during reward-based behaviors. *Neuron* 97, 494–510. <https://doi.org/10.1016/j.neuron.2018.01.006>.
102. Kano, M., Ohno-Shosaku, T., Hashimoto, Y., Uchigashima, M., and Watanabe, M. (2009). Endocannabinoid-mediated control of synaptic transmission. *Physiol. Rev.* 89, 309–380. <https://doi.org/10.1152/physrev.00019.2008>.
103. Glass, M., Dragunow, M., and Faull, R.L.M. (1997). 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.
104. Tsou, K., Brown, S., Sañudo-Peña, M.C., Mackie, K., and Walker, J.M. (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* 83, 393–411.
105. Davis, M.I., Crittenden, J.R., Feng, A.Y., Kupferschmidt, D.A., Naydenov, A., Stella, N., Graybiel, A.M., and Lovinger, D.M. (2018). The cannabinoid-1 receptor is abundantly expressed in striatal striosomes and striosome-dendron bouquets of the substantia nigra. *PLoS One* 13, e0191436. <https://doi.org/10.1371/journal.pone.0191436>.
106. Hohmann, A.G., and Herkenham, M. (2000). Localization of cannabinoid CB1 receptor mRNA in neuronal subpopulations of rat striatum: A double-label in situ hybridization study. *Synapse* 37, 71–80.
107. Hu, S.S.-J., and Mackie, K. (2015). Distribution of the endocannabinoid system in the central nervous system. *Handb. Exp. Pharmacol.* 231, 59–93.
108. Uchigashima, M., Narushima, M., Fukaya, M., Katona, I., Kano, M., and Watanabe, M. (2007). Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. *J. Neurosci.* 27, 3663–3676. <https://doi.org/10.1523/JNEUROSCI.0448-07.2007>.
109. Yin, H.H., and Lovinger, D.M. (2006). Frequency-specific and D2 receptor-mediated inhibition of glutamate release by retrograde endocannabinoid signaling. *Proc. Natl. Acad. Sci. USA* 103, 8251–8256. <https://doi.org/10.1073/pnas.0510797103>.
110. Bamford, N.S., Zhang, H., Schmitz, Y., Wu, N.P., Cepeda, C., Levine, M.S., Schmauss, C., Zakharenko, S.S., Zablow, L., and Sulzer, D. (2004). Heterosynaptic dopamine neurotransmission selects sets of corticostriatal terminals. *Neuron* 42, 653–663.
111. Pickel, V.M., Chan, J., Kearn, C.S., and Mackie, K. (2006). Targeting dopamine D2 and cannabinoid-1 (CB1) receptors in rat nucleus accumbens. *J. Comp. Neurol.* 495, 299–313. <https://doi.org/10.1002/cne.20881>.
112. Marcellino, D., Carriba, P., Filip, M., Borgkvist, A., Frankowska, M., Bel-lido, I., Tanganelli, S., Müller, C.E., Fisone, G., Lluis, C., et al. (2008). Antagonistic cannabinoid CB1/dopamine D2 receptor interactions in striatal CB1/D2 heteromers. A combined neurochemical and behavioral analysis. *Neuropharmacology* 54, 815–823. <https://doi.org/10.1016/j.neuropharm.2007.12.011>.
113. Nagai, H., Egashira, N., Sano, K., Ogata, A., Mizuki, A., Mishima, K., Iwasaki, K., Shoyama, Y., Nishimura, R., and Fujiwara, M. (2006). Antipsychotics improve $\Delta 9$ -tetrahydrocannabinol-induced impairment of the prepulse inhibition of the startle reflex in mice. *Pharmacol. Biochem. Behav.* 84, 330–336.
114. Marques, A.M., Macena, M.V., Cardoso, A.R., Hammes, C.S.O., Pinheiro, F.M.L., Castro, N.G., and Neves, G.A. (2020). Effects of combined 5-HT2A and cannabinoid receptor modulation on a schizophrenia-related prepulse inhibition deficit in mice. *Psychopharmacology* 237, 1643–1655.
115. Barnard, I.L., Onofrychuk, T.J., Sandini, T.M., McElroy, D.L., Zagzoog, A., Roebuck, A.J., Austin-Scott, F.V., Laprairie, R.B., and Howland, J.G. (2022). The effects of acute cannabis smoke or $\Delta 9$ -THC injections on the trial-unique, nonmatching-to-location and five-choice serial reaction time tasks in male Long-Evans rats. *Neurobiol. Learn. Mem.* 192, 107624. <https://doi.org/10.1016/j.nlm.2022.107624>.
116. Gilman, J.M., Schmitt, W.A., Potter, K., Kendzior, B., Pachas, G.N., Hickley, S., Makary, M., Huestis, M.A., and Evins, A.E. (2022). Identification of

- Δ^9 -tetrahydrocannabinol (THC) impairment using functional brain imaging. *Neuropsychopharmacology* 47, 944–952.
117. Fortin, D.A., and Levine, E.S. (2007). Differential effects of endocannabinoids on glutamatergic and GABAergic inputs to layer 5 pyramidal neurons. *Cereb. Cortex* 17, 163–174.
118. Pouliu, N., Delis, F., Brakatselos, C., Polissidis, A., Koutmani, Y., Kokras, N., Dalla, C., Politis, P.K., and Antoniou, K. (2021). Detrimental effects of adolescent escalating low-dose Δ^9 -tetrahydrocannabinol leads to a specific bio-behavioural profile in adult male rats. *Br. J. Pharmacol.* 178, 1722–1736.
119. Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S.C., Cascio, M.G., Gutiérrez, S.O., van der Stelt, M., et al. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 302, 84–88.
120. Den Boon, F.S., Werkman, T.R., Schaafsma-Zhao, Q., Houthuijs, K., Vitalis, T., Kruse, C.G., Wadman, W.J., and Chameau, P. (2015). Activation of type-1 cannabinoid receptor shifts the balance between excitation and inhibition towards excitation in layer II/III pyramidal neurons of the rat pre- limbic cortex. *Pflugers Arch.* 467, 1551–1564.
121. Caballero, A., Granberg, R., and Tseng, K.Y. (2016). Mechanisms contributing to prefrontal cortex maturation during adolescence. *Neurosci. Biobehav. Rev.* 70, 4–12.
122. Lui, J.H., Nguyen, N.D., Grutzner, S.M., Darmanis, S., Peixoto, D., Wagner, M.J., Allen, W.E., Kebschull, J.M., Richman, E.B., Ren, J., et al. (2021). Differential encoding in prefrontal cortex projection neuron classes across cognitive tasks. *Cell* 184, 489–506.e26.
123. Morgunova, A., and Flores, C. (2021). *Seminars in Cell & Developmental Biology* (Elsevier) 118, 1–172.
124. Tinklenberg, J.R., Melges, F.T., Hollister, L.E., and Gillespie, H.K. (1970). Marijuana and immediate memory. *Nature* 226, 1171–1172.
125. Adam, K.C.S., Doss, M.K., Pabon, E., Vogel, E.K., and de Wit, H. (2020). Δ^9 -tetrahydrocannabinol (THC) impairs visual working memory performance: a randomized crossover trial. *Neuropsychopharmacology* 45, 1807–1816.
126. Varvel, S.A., Hamm, R.J., Martin, B.R., and Lichtman, A.H. (2001). Differential effects of Δ^9 -THC on spatial reference and working memory in mice. *Psychopharmacology* 157, 142–150.
127. Cha, Y.M., Jones, K.H., Kuhn, C.M., Wilson, W.A., and Swartzwelder, H.S. (2007). Sex differences in the effects of Δ^9 -tetrahydrocannabinol on spatial learning in adolescent and adult rats. *Behav. Pharmacol.* 18, 563–569.
128. Chen, R., Zhang, J., Fan, N., Teng, Z.Q., Wu, Y., Yang, H., Tang, Y.P., Sun, H., Song, Y., and Chen, C. (2013). Δ^9 -THC-caused synaptic and memory impairments are mediated through COX-2 signaling. *Cell* 155, 1154–1165.
129. Robbe, D., Montgomery, S.M., Thome, A., Rueda-Orozco, P.E., McNaughton, B.L., and Buzsáki, G. (2006). Cannabinoids reveal importance of spike timing coordination in hippocampal function. *Nat. Neurosci.* 9, 1526–1533. <https://doi.org/10.1038/nn1801>.
130. Hebert-Chatelain, E., Desprez, T., Serrat, R., Bellocchio, L., Soria-Gomez, E., Busquets-Garcia, A., Pagano Zottola, A.C., Delamarre, A., Canich, A., Vincent, P., et al. (2016). A cannabinoid link between mitochondria and memory. *Nature* 539, 555–559.
131. Maroso, M., Szabo, G.G., Kim, H.K., Alexander, A., Bui, A.D., Lee, S.H., Lutz, B., and Soltesz, I. (2016). Cannabinoid control of learning and memory through HCN channels. *Neuron* 89, 1059–1073.
132. Busquets-Garcia, A., Gomis-González, M., Salgado-Mendialdúa, V., Gallera-López, L., Puighermanal, E., Martín-García, E., Maldonado, R., and Ozaita, A. (2018). Hippocampal protein kinase C signaling mediates the short-term memory impairment induced by Δ^9 -tetrahydrocannabinol. *Neuropsychopharmacology* 43, 1021–1031.
133. Hampson, R.E., and Deadwyler, S.A. (2000). Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. *J. Neurosci.* 20, 8932–8942.
134. Tournier, B.B., and Ginovart, N. (2014). Repeated but not acute treatment with Δ^9 -tetrahydrocannabinol disrupts prepulse inhibition of the acoustic startle: reversal by the dopamine $D_{2/3}$ receptor antagonist haloperidol. *Eur. Neuropsychopharmacol.* 24, 1415–1423.
135. Dar, M.S. (2000). Cerebellar CB1 receptor mediation of Δ^9 -THC-induced motor incoordination and its potentiation by ethanol and modulation by the cerebellar adenosinergic A1 receptor in the mouse. *Brain Res.* 864, 186–194.
136. Saravia, R., Ten-Blanco, M., Julià-Hernández, M., Gagliano, H., Andero, R., Armario, A., Maldonado, R., and Berrendero, F. (2019). Concomitant THC and stress adolescent exposure induces impaired fear extinction and related neurobiological changes in adulthood. *Neuropharmacology* 144, 345–357.
137. Calabrese, E.J., and Rubio-Casillas, A. (2018). Biphasic effects of THC in memory and cognition. *Eur. J. Clin. Invest.* 48, e12920.
138. D'Souza, D.C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y.T., Braley, G., Gueorguieva, R., and Krystal, J.H. (2004). The psychotomimetic effects of intravenous Δ^9 -tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology* 29, 1558–1572.
139. Mennis, J., Stahler, G.J., and McKeon, T.P. (2021). Young adult cannabis use disorder treatment admissions declined as past month cannabis use increased in the U.S.: an analysis of states by year, 2008–2017. *Addict. Behav.* 123, 107049. <https://doi.org/10.1016/j.addbeh.2021.107049>.
140. Antonia, M., and Viviana, T. (2022). Behavioral consequences of pre/perinatal cannabis exposure. *Cannabis and the Developing Brain* (Elsevier).
141. Roberto, F., and Miriam, M. (2022). Effects of prenatal THC exposure on the mesolimbic dopamine system: unveiling an endophenotype of sensory information processing deficits. *Cannabis and the Developing Brain* (Elsevier).
142. Di Marzo, V., Stella, N., and Zimmer, A. (2015). Endocannabinoid signaling and the deteriorating brain. *Nat. Rev. Neurosci.* 16, 30–42. <https://doi.org/10.1038/nrn3876>.
143. Albaugh, M.D., Ottino-Gonzalez, J., Sidwell, A., Lepage, C., Juliano, A., Owens, M.M., Chaarani, B., Spechler, P., Fontaine, N., Rioux, P., et al. (2021). Association of cannabis use during adolescence with neurodevelopment. *JAMA Psychiatry* 78, 1–11.
144. Barrington-Trimis, J.L., Cho, J., Ewusi-Boisvert, E., Hasin, D., Unger, J.B., Miech, R.A., and Leventhal, A.M. (2020). Risk of persistence and progression of use of 5 cannabis products after experimentation among adolescents. *JAMA Netw. Open* 3, e1919792.
145. Hammond, C.J., Chaney, A., Hendrickson, B., and Sharma, P. (2020). Cannabis use among US adolescents in the era of marijuana legalization: a review of changing use patterns, comorbidity, and health correlates. *Int. Rev. Psychiatry* 32, 221–234.
146. Knapp, A.A., Lee, D.C., Borodovsky, J.T., Auty, S.G., Gabrielli, J., and Budney, A.J. (2019). Emerging trends in cannabis administration among adolescent cannabis users. *J. Adolesc. Health* 64, 487–493.
147. Gunn, R.L., Aston, E.R., Sokolovsky, A.W., White, H.R., and Jackson, K.M. (2020). Complex cannabis use patterns: associations with cannabis consequences and cannabis use disorder symptomatology. *Addict. Behav.* 105, 106329. <https://doi.org/10.1016/j.addbeh.2020.106329>.
148. Arterberry, B.J., Treloar Padovano, H.T., Foster, K.T., Zucker, R.A., and Hicks, B.M. (2019). Higher average potency across the United States is associated with progression to first cannabis use disorder symptom. *Drug Alcohol Depend.* 195, 186–192.
149. Camchong, J., Lim, K.O., and Kumra, S. (2017). Adverse effects of cannabis on adolescent brain development: a longitudinal study. *Cereb. Cortex* 27, 1922–1930.
150. Schoeler, T., Ferris, J., and Winstock, A.R. (2022). Rates and correlates of cannabis-associated psychotic symptoms in over 230,000 people who use cannabis. *Transl. Psychiatry* 12, 369.

151. Betz, L.T., Penzel, N., and Kambeitz, J. (2022). A network approach to relationships between cannabis use characteristics and psychopathology in the general population. *Sci. Rep.* 12, 7163.
152. Morrison, P.D., Zois, V., McKeown, D.A., Lee, T.D., Holt, D.W., Powell, J.F., Kapur, S., and Murray, R.M. (2009). The acute effects of synthetic intravenous Δ^9 -tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychol. Med.* 39, 1607–1616.
153. Van der Steur, S.J., Batalla, A., and Bossong, M.G. (2020). Factors moderating the association between cannabis use and psychosis risk: a systematic review. *Brain Sci.* 10, 97.
154. Di Forti, M., Sallis, H., Allegrì, F., Trotta, A., Ferraro, L., Stilo, S.A., Marconi, A., La Cascia, C., Reis Marques, T., Pariante, C., et al. (2014). Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. *Schizophr. Bull.* 40, 1509–1517.
155. Di Forti, M., Quattrone, D., Freeman, T.P., Tripoli, G., Gayer-Anderson, C., Quigley, H., Rodriguez, V., Jongsma, H.E., Ferraro, L., La Cascia, C., et al. (2019). The contribution of cannabis use to variation in the incidence of psychotic disorder across Europe (EU-GEI): a multicentre case-control study. *Lancet Psychiatry* 6, 427–436.
156. Pierre, J.M., Gandal, M., and Son, M. (2016). Cannabis-induced psychosis associated with high potency “wax dabs”. *Schizophr. Res.* 172, 211–212.
157. Gobbi, G., Atkin, T., Zytynski, T., Wang, S., Askari, S., Boruff, J., Ware, M., Marmorstein, N., Cipriani, A., Dendukuri, N., and Mayo, N. (2019). Association of cannabis use in adolescence and risk of depression, anxiety, and suicidality in young adulthood: a systematic review and meta-analysis. *JAMA Psychiatry* 76, 426–434.
158. Jouroukhin, Y., Zhu, X., Shevelkin, A.V., Hasegawa, Y., Abazyan, B., Saito, A., Pevsner, J., Kamiya, A., and Pletnikov, M.V. (2019). Adolescent Δ^9 -tetrahydrocannabinol exposure and astrocyte-specific genetic vulnerability converge on nuclear factor- κ B–cyclooxygenase-2 signaling to impair memory in adulthood. *Biol. Psychiatry* 85, 891–903.
159. Chen, H.-T., and Mackie, K. (2020). Adolescent Δ^9 -tetrahydrocannabinol exposure selectively impairs working memory but not several other mPFC-mediated behaviors. *Front. Psychiatry* 11, 576214.
160. Hempel, B.J., Wakeford, A.G., Clasen, M.M., Friar, M.A., and Riley, A.L. (2016). Delta-9-tetrahydrocannabinol (THC) history fails to affect THC's ability to induce place preferences in rats. *Pharmacol. Biochem. Behav.* 144, 1–6.
161. Flores, Á., Maldonado, R., and Berrendero, F. (2020). THC exposure during adolescence does not modify nicotine reinforcing effects and relapse in adult male mice. *Psychopharmacology* 237, 801–809.
162. Szkudlarek, H.J., Desai, S.J., Renard, J., Pereira, B., Norris, C., Jobson, C.E.L., Rajakumar, N., Allman, B.L., and Laviolette, S.R. (2019). Δ^9 -tetrahydrocannabinol and cannabidiol produce dissociable effects on prefrontal cortical executive function and regulation of affective behaviors. *Neuropsychopharmacology* 44, 817–825.
163. VanRyzin, J.W., Marquardt, A.E., Argue, K.J., Vecchiarelli, H.A., Ashton, S.E., Arambula, S.E., Hill, M.N., and McCarthy, M.M. (2019). Microglial phagocytosis of newborn cells is induced by endocannabinoids and sculpts sex differences in juvenile rat social play. *Neuron* 102, 435–449.e6.
164. Lee, H.L., Jung, K.M., Fotio, Y., Squire, E., Palese, F., Lin, L., Torrens, A., Ahmed, F., Mabou Tagne, A., Ramirez, J., et al. (2022). Frequent low-dose Δ^9 -tetrahydrocannabinol in adolescence disrupts microglia homeostasis and disables responses to microbial infection and social stress in young adulthood. *Biol. Psychiatry* 92, 845–860.
165. Erica, Z., Cristina, M., Marina, G., RubinoTiziana, and Daniela, P. (2022). Correlates and consequences of cannabinoid exposure on adolescent brain remodeling: Focus on glial cells and epigenetics. *Cannabis and the Developing Brain* (Elsevier).
166. Parsons, L.H., and Hurd, Y.L. (2015). Endocannabinoid signalling in reward and addiction. *Nat. Rev. Neurosci.* 16, 579–594.
167. Ellgren, M., Spano, S.M., and Hurd, Y.L. (2007). Adolescent cannabis exposure alters opiate intake and opioid limbic neuronal populations in adult rats. *Neuropsychopharmacology* 32, 607–615.
168. Tomasiwicz, H.C., Jacobs, M.M., Wilkinson, M.B., Wilson, S.P., Nestler, E.J., and Hurd, Y.L. (2012). Proenkephalin mediates the enduring effects of adolescent cannabis exposure associated with adult opiate vulnerability. *Biol. Psychiatry* 72, 803–810.
169. Lecca, D., Scifo, A., Pisanu, A., Valentini, V., Piras, G., Sil, A., Cadoni, C., and Di Chiara, G. (2020). Adolescent cannabis exposure increases heroin reinforcement in rats genetically vulnerable to addiction. *Neuropharmacology* 166, 107974. <https://doi.org/10.1016/j.neuropharm.2020.107974>.
170. Friedman, A.L., Meurice, C., and Jutkiewicz, E.M. (2019). Effects of adolescent Δ^9 -tetrahydrocannabinol exposure on the behavioral effects of cocaine in adult Sprague–Dawley rats. *Exp. Clin. Psychopharmacol.* 27, 326–337.
171. Cheer, J.F., Wassum, K.M., Sombers, L.A., Heien, M.L., Ariansen, J.L., Aragona, B.J., Phillips, P.E., and Wightman, R.M. (2007). Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. *J. Neurosci.* 27, 791–795.
172. Corbillé, A.G., Valjent, E., Marsicano, G., Ledent, C., Lutz, B., Hervé, D., and Girault, J.A. (2007). Role of cannabinoid type 1 receptors in locomotor activity and striatal signaling in response to psychostimulants. *J. Neurosci.* 27, 6937–6947.
173. Covey, D.P., Bunner, K.D., Schuweiler, D.R., Cheer, J.F., and Garris, P.A. (2016). Amphetamine elevates nucleus accumbens dopamine via an action potential-dependent mechanism that is modulated by endocannabinoids. *Eur. J. Neurosci.* 43, 1661–1673.
174. Deng, L., Viray, K., Singh, S., Cravatt, B., and Stella, N. (2022). ABHD6 controls amphetamine-stimulated hyperlocomotion: involvement of CB1 receptors. *Cannabis Cannabinoid Res.* 7, 188–198.
175. English, A., Land, B.B., and Stella, N. (2022). Impact of adolescent THC exposure on later adulthood: Focus on mesocorticolimbic function and behaviors. *Cannabis and the Developing Brain* (Elsevier).
176. Pickel, V.M., Bourie, F., Chan, J., Mackie, K., Lane, D.A., and Wang, G. (2020). Chronic adolescent exposure to Δ^9 -tetrahydrocannabinol decreases NMDA current and extrasynaptic plasmalemmal density of NMDA GluN1 subunits in the prelimbic cortex of adult male mice. *Neuropsychopharmacology* 45, 374–383.
177. Renard, J., Szkudlarek, H.J., Kramar, C.P., Jobson, C.E.L., Moura, K., Rushlow, W.J., and Laviolette, S.R. (2017). Adolescent THC exposure causes enduring prefrontal cortical disruption of GABAergic inhibition and dysregulation of sub-cortical dopamine function. *Sci. Rep.* 7, 11420.
178. Renard, J., Norris, C., Rushlow, W., and Laviolette, S.R. (2017). Neuronal and molecular effects of cannabidiol on the mesolimbic dopamine system: implications for novel schizophrenia treatments. *Neurosci. Biobehav. Rev.* 75, 157–165.
179. Rubino, T., Prini, P., Piscitelli, F., Zamberletti, E., Trusel, M., Melis, M., Sagheddu, C., Ligresti, A., Tonini, R., Di Marzo, V., et al. (2015). Adolescent exposure to THC in female rats disrupts developmental changes in the prefrontal cortex. *Neurobiol. Dis.* 73, 60–69.
180. Buxbaum, D.M. (1972). Analgesic activity of 9-tetrahydrocannabinol in the rat and mouse. *Psychopharmacologia* 25, 275–280.
181. Fisher, E., Eccleston, C., Degenhardt, L., Finn, D.P., Finnerup, N.B., Gilron, I., Haroutounian, S., Krane, E., Rice, A.S.C., Rowbotham, M., et al. (2019). Cannabinoids, cannabis, and cannabis-based medicine for pain management: a protocol for an overview of systematic reviews and a systematic review of randomised controlled trials. *PAIN Rep.* 4, e741.
182. Almog, S., Aharon-Peretz, J., Vulfsons, S., Ogintz, M., Abalia, H., Lupo, T., Hayon, Y., and Eisenberg, E. (2020). The pharmacokinetics, efficacy, and safety of a novel selective-dose cannabis inhaler in patients with chronic pain: A randomized, double-blinded, placebo-controlled trial. *Eur. J. Pain* 24, 1505–1516.
183. Soliman, N., Haroutounian, S., Hohmann, A.G., Krane, E., Liao, J., Macleod, M., Segelcke, D., Sena, C., Thomas, J., Vollert, J., et al. (2021).

Systematic review and meta-analysis of cannabinoids, cannabis-based medicines, and endocannabinoid system modulators tested for antinociceptive effects in animal models of injury-related or pathological persistent pain. *Pain* 162, S26–S44.

184. Finn, D.P., Haroutounian, S., Hohmann, A.G., Krane, E., Soliman, N., and Rice, A.S.C. (2021). Cannabinoids, the endocannabinoid system, and pain: a review of preclinical studies. *Pain* 162, S5–S25.
185. Gutierrez, A., Nguyen, J.D., Creehan, K.M., Javadi-Paydar, M., Grant, Y., and Taffe, M.A. (2022). Effects of combined THC and heroin vapor inhalation in rats. *Psychopharmacology* 239, 1321–1335.
186. Deng, L., Guindon, J., Cornett, B.L., Makriyannis, A., Mackie, K., and Hohmann, A.G. (2015). Chronic cannabinoid receptor 2 activation reverses paclitaxel neuropathy without tolerance or cannabinoid receptor 1-dependent withdrawal. *Biol. Psychiatry* 77, 475–487. <https://doi.org/10.1016/j.biopsych.2014.04.009>.
187. Gutierrez, T., Crystal, J.D., Zvonok, A.M., Makriyannis, A., and Hohmann, A.G. (2011). Self-medication of a cannabinoid CB2 agonist in an animal model of neuropathic pain. *Pain* 152, 1976–1987.
188. Lee, M.T., Mackie, K., and Chiou, L.C. (2021). Alternative pain management via endocannabinoids in the time of the opioid epidemic: Peripheral neuromodulation and pharmacological interventions. *Br. J. Pharmacol.* <https://doi.org/10.1111/bph.15771>.
189. Belendiuk, K.A., Babson, K.A., Vandrey, R., and Bonn-Miller, M.O. (2015). Cannabis species and cannabinoid concentration preference among sleep-disturbed medicinal cannabis users. *Addict. Behav.* 50, 178–181.
190. Fabbri, M., Beracci, A., Martoni, M., Meneo, D., Tonetti, L., and Natale, V. (2021). Measuring subjective sleep quality: a review. *Int. J. Environ. Res. Public Health* 18, 1082.
191. Prasad, B., Radulovacki, M.G., and Carley, D.W. (2013). Proof of concept trial of dronabinol in obstructive sleep apnea. *Front. Psychiatry* 4, 1.
192. Kuhathasan, N., Dufort, A., MacKillop, J., Gottschalk, R., Minuzzi, L., and Frey, B.N. (2019). The use of cannabinoids for sleep: A critical review on clinical trials. *Exp. Clin. Psychopharmacol.* 27, 383–401.
193. Bonn-Miller, M.O., Babson, K.A., and Vandrey, R. (2014). Using cannabis to help you sleep: heightened frequency of medical cannabis use among those with PTSD. *Drug Alcohol Depend.* 136, 162–165.
194. Budney, A.J., Vandrey, R.G., Hughes, J.R., Thostenson, J.D., and Bursac, Z. (2008). Comparison of cannabis and tobacco withdrawal: severity and contribution to relapse. *J. Subst. Abuse Treat.* 35, 362–368.
195. Monti, J.M., and Pandi-Perumal, S.R. (2022). Clinical management of sleep and sleep disorders with cannabis and cannabinoids: implications to practicing psychiatrists. *Clin. Neuropharmacol.* 45, 27–31.
196. Roitman, P., Mechoulam, R., Cooper-Kazaz, R., and Shalev, A. (2014). Preliminary, open-label, pilot study of add-on oral Δ 9-tetrahydrocannabinol in chronic post-traumatic stress disorder. *Clin. Drug Investig.* 34, 587–591.
197. Fraser, G.A. (2009). The use of a synthetic cannabinoid in the management of treatment-resistant nightmares in posttraumatic stress disorder (PTSD). *CNS Neurosci. Ther.* 15, 84–88.
198. Jetly, R., Heber, A., Fraser, G., and Boisvert, D. (2015). The efficacy of nabilone, a synthetic cannabinoid, in the treatment of PTSD-associated nightmares: a preliminary randomized, double-blind, placebo-controlled cross-over design study. *Psychoneuroendocrinology* 57, 585–588.
199. Kesner, A.J., Mateo, Y., Abrahao, K.P., Ramos-Maciel, S., Pava, M.J., Gracias, A.L., Paulsen, R.T., Carlson, H.B., and Lovinger, D.M. (2022). Changes in striatal dopamine release, sleep, and behavior during spontaneous Δ 9-tetrahydrocannabinol abstinence in male and female mice. *Neuropsychopharmacology* 47, 1537–1549.
200. Silvani, A., Berteotti, C., Bastianini, S., Lo Martire, V., Mazza, R., Pagotto, U., Quarta, C., and Zoccoli, G. (2014). Multiple sleep alterations in mice lacking cannabinoid type 1 receptors. *PLoS ONE* 9, e89432.
201. Corcoran, M.E., McCaughan, J.A., Jr., and Wada, J.A. (1973). Acute antiepileptic effects of 9-tetrahydrocannabinol in rats with kindled seizures. *Exp. Neurol.* 40, 471–483.
202. Wallace, M.J., Blair, R.E., Falenski, K.W., Martin, B.R., and DeLorenzo, R.J. (2003). The endogenous cannabinoid system regulates seizure frequency and duration in a model of temporal lobe epilepsy. *J. Pharmacol. Exp. Ther.* 307, 129–137.
203. Krook-Magnuson, E., Armstrong, C., Bui, A., Lew, S., Oijala, M., and Soltesz, I. (2015). In vivo evaluation of the dentate gate theory in epilepsy. *J. Physiol.* 593, 2379–2388.
204. Dengler, C.G., and Coulter, D.A. (2016). Normal and epilepsy-associated pathologic function of the dentate gyrus. *Prog. Brain Res.* 226, 155–178.
205. Shen, M., and Thayer, S.A. (1999). Delta9-tetrahydrocannabinol acts as a partial agonist to modulate glutamatergic synaptic transmission between rat hippocampal neurons in culture. *Mol. Pharmacol.* 55, 8–13.
206. Whalley, B.J., Lin, H., Bell, L., Hill, T., Patel, A., Gray, R.A., Elizabeth Roberts, C., Devinsky, O., Bazet, M., Williams, C.M., and Stephens, G.J. (2019). Species-specific susceptibility to cannabis-induced convulsions. *Br. J. Pharmacol.* 176, 1506–1523.
207. Huizenga, M.N., Fureman, B.E., Soltesz, I., and Stella, N. (2018). Proceedings of the Epilepsy Foundation's 2017 Cannabinoids in Epilepsy Therapy Workshop. *Epilepsy Behav.* 85, 237–242.
208. Zareie, P., Sadegh, M., Palizvan, M.R., and Moradi-Chameh, H. (2018). Anticonvulsive effects of endocannabinoids; an investigation to determine the role of regulatory components of endocannabinoid metabolism in the pentylenetetrazol induced tonic-clonic seizures. *Metab. Brain Dis.* 33, 939–948.
209. Von Rüden, E.L., Bogdanovic, R.M., Wotjak, C.T., and Potschka, H. (2015). Inhibition of monoacylglycerol lipase mediates a cannabinoid 1-receptor dependent delay of kindling progression in mice. *Neurobiol. Dis.* 77, 238–245.
210. Roebuck, A.J., Greba, Q., Smolyakova, A.M., Alaverdashvili, M., Marks, W.N., Garai, S., Baglot, S.L., Petrie, G., Cain, S.M., Snutch, T.P., et al. (2021). Positive allosteric modulation of type 1 cannabinoid receptors reduces spike-and-wave discharges in Genetic Absence epilepsy Rats from Strasbourg. *Neuropharmacology* 190, 108553. <https://doi.org/10.1016/j.neuropharm.2021.108553>.
211. van Amerongen, G., Kanhai, K., Baakman, A.C., Heuberger, J., Klaassen, E., Beumer, T.L., Strijers, R.L.M., Killestein, J., van Gerven, J., Cohen, A., et al. (2018). Effects on spasticity and neuropathic pain of an oral formulation of Δ 9-tetrahydrocannabinol in patients with progressive multiple sclerosis. *Clin. Ther.* 40, 1467–1482.
212. Di Marzo, V., and Centonze, D. (2015). Placebo effects in a multiple sclerosis spasticity enriched clinical trial with the oromucosal cannabinoid spray (THC/CBD): dimension and possible causes. *CNS Neurosci. Ther.* 21, 215–221.
213. Maresz, K., Pryce, G., Ponomarev, E.D., Marsicano, G., Croxford, J.L., Shriver, L.P., Ledent, C., Cheng, X., Carrier, E.J., Mann, M.K., et al. (2007). Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nat. Med.* 13, 492–497. <https://doi.org/10.1038/nm1561>.
214. Lyman, W.D., Sonett, J.R., Brosnan, C.F., Elkin, R., and Bornstein, M.B. (1989). Δ 9-tetrahydrocannabinol: a novel treatment for experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 23, 73–81.
215. Baker, D., Pryce, G., Croxford, J.L., Brown, P., Pertwee, R.G., Huffman, J.W., and Layward, L. (2000). Cannabinoids control spasticity and tremor in a multiple sclerosis model. *Nature* 404, 84–87.
216. Pryce, G., Ahmed, Z., Hankey, D.J., Jackson, S.J., Croxford, J.L., Pocock, J.M., Ledent, C., Petzold, A., Thompson, A.J., Giovannoni, G., et al. (2003). Cannabinoids inhibit neurodegeneration in models of multiple sclerosis. *Brain* 126, 2191–2202.
217. López-Sendón Moreno, J.L., García Caldentey, J., Trigo Cubillo, P., Ruiz Romero, C., García Ribas, G., Alonso Arias, M.A., García de Yébenes, M.J., Tolón, R.M., Galve-Roperh, I., Sagredo, O., et al. (2016). A

- double-blind, randomized, cross-over, placebo-controlled, pilot trial with Sativex in Huntington's disease. *J. Neurol.* 263, 1390–1400.
218. Naydenov, A.V., Sepers, M.D., Swinney, K., Raymond, L.A., Palmiter, R.D., and Stella, N. (2014). Genetic rescue of CB1 receptors on medium spiny neurons prevents loss of excitatory striatal synapses but not motor impairment in HD mice. *Neurobiol. Dis.* 71, 140–150. <https://doi.org/10.1016/j.nbd.2014.08.009>.
219. Woodman, B., Butler, R., Landles, C., Lupton, M.K., Tse, J., Hockly, E., Moffitt, H., Sathasivam, K., and Bates, G.P. (2007). The Hdh(Q150/Q150) knock-in mouse model of HD and the R6/2 exon 1 model develop comparable and widespread molecular phenotypes. *Brain Res. Bull.* 72, 83–97. <https://doi.org/10.1016/j.brainresbull.2006.11.004>.
220. Horne, E.A., Coy, J., Swinney, K., Fung, S., Cherry, A.E., Marrs, W.R., Naydenov, A.V., Lin, Y.H., Sun, X., Keene, C.D., et al. (2013). Downregulation of cannabinoid receptor 1 from neuropeptide Y interneurons in the basal ganglia of patients with Huntington's disease and mouse models. *Eur. J. Neurosci.* 37, 429–440. <https://doi.org/10.1111/ejn.12045>.
221. Ruiz-Calvo, A., Bajo-Grañeras, R., Maroto, I.B., Zian, D., Grabner, G.F., García-Taboada, E., Resel, E., Zechner, R., Zimmermann, R., Ortega-Gutiérrez, S., et al. (2019). Astroglial monoacylglycerol lipase controls mutant huntingtin-induced damage of striatal neurons. *Neuropharmacology* 150, 134–144.
222. Chiaroni, A., Bellochio, L., Blázquez, C., Resel, E., Soria-Gómez, E., Cannich, A., Ferrero, J.J., Sagredo, O., Benito, C., Romero, J., et al. (2014). A restricted population of CB1 cannabinoid receptors with neuroprotective activity. *Proc. Natl. Acad. Sci. USA* 111, 8257–8262.
223. Sagredo, O., Pazos, M.R., Satta, V., Ramos, J.A., Pertwee, R.G., and Fernández-Ruiz, J. (2011). Neuroprotective effects of phytocannabinoid-based medicines in experimental models of Huntington's disease. *J. Neurosci. Res.* 89, 1509–1518. <https://doi.org/10.1002/jnr.22682>.
224. Liebling, J.P., Clarkson, N.J., Gibbs, B.W., Yates, A.S., and O'Sullivan, S.E. (2022). An analysis of over-the-counter cannabidiol products in the United Kingdom. *Cannabis Cannabinoid Res.* 7, 207–213.
225. Spindle, T.R., Bonn-Miller, M.O., and Vandrey, R. (2019). Changing landscape of cannabis: novel products, formulations, and methods of administration. *Curr. Opin. Psychol.* 30, 98–102.
226. Bergeria, C.L., Spindle, T.R., Cone, E.J., Sholler, D., Goffi, E., Mitchell, J.M., Winecker, R.E., Bigelow, G.E., Flegel, R., and Vandrey, R. (2022). Pharmacokinetic profile of Δ^9 -tetrahydrocannabinol, cannabidiol and metabolites in blood following vaporization and oral ingestion of cannabidiol products. *J. Anal. Toxicol.* 46, 583–591.
227. Devinsky, O., Kraft, K., Rusch, L., Fein, M., and Leone-Bay, A. (2021). Improved bioavailability with dry powder cannabidiol inhalation: A phase 1 clinical study. *J. Pharm. Sci.* 110, 3946–3952.
228. Deiana, S., Watanabe, A., Yamasaki, Y., Amada, N., Arthur, M., Fleming, S., Woodcock, H., Dorward, P., Pigliacampo, B., Close, S., et al. (2012). Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarin (CBDV), Δ^9 -tetrahydrocannabinol (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. *Psychopharmacology* 219, 859–873.
229. Bornheim, L.M., and Grillo, M.P. (1998). Characterization of cytochrome P450 3A inactivation by cannabidiol: possible involvement of cannabidiol-hydroxyquinone as a P450 inactivator. *Chem. Res. Toxicol.* 11, 1209–1216.
230. Anderson, L.L., Absalom, N.L., Abelev, S.V., Low, I.K., Doohan, P.T., Martin, L.J., Chebib, M., McGregor, I.S., and Arnold, J.C. (2019). Co-administered cannabidiol and clobazam: preclinical evidence for both pharmacodynamic and pharmacokinetic interactions. *Epilepsia* 60, 2224–2234.
231. Geffrey, A.L., Pollack, S.F., Bruno, P.L., and Thiele, E.A. (2015). Drug-drug interaction between clobazam and cannabidiol in children with refractory epilepsy. *Epilepsia* 56, 1246–1251.
232. Thomas, B.F., Gilliam, A.F., Burch, D.F., Roche, M.J., and Seltzman, H.H. (1998). Comparative receptor binding analyses of cannabinoid agonists and antagonists. *J. Pharmacol. Exp. Ther.* 285, 285–292.
233. Jakowiecki, J., Abel, R., Orzel, U., Pasznik, P., Preissner, R., and Filipek, S. (2021). Allosteric modulation of the CB1 cannabinoid receptor by cannabidiol—A molecular modeling study of the N-terminal domain and the allosteric-orthosteric coupling. *Molecules* 26, 2456.
234. Straiker, A., Dvorakova, M., Zimmowitch, A., and Mackie, K. (2018). Cannabidiol inhibits endocannabinoid signaling in autaptic hippocampal neurons. *Mol. Pharmacol.* 94, 743–748.
235. Laprairie, R.B., Bagher, A.M., Kelly, M.E., Dupré, D.J., and Denovan-Wright, E.M. (2014). Type 1 cannabinoid receptor ligands display functional selectivity in a cell culture model of striatal medium spiny projection neurons. *J. Biol. Chem.* 289, 24845–24862. <https://doi.org/10.1074/jbc.M114.557025>.
236. Laprairie, R.B., Bagher, A.M., Kelly, M.E., and Denovan-Wright, E.M. (2015). Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. *Br. J. Pharmacol.* 172, 4790–4805.
237. Hill, J.D., Zuluaga-Ramirez, V., Gajghate, S., Winfield, M., and Persidsky, Y. (2018). Activation of GPR55 increases neural stem cell proliferation and promotes early adult hippocampal neurogenesis. *Br. J. Pharmacol.* 175, 3407–3421.
238. Ross, R.A. (2009). The enigmatic pharmacology of GPR55. *Trends Pharmacol. Sci.* 30, 156–163.
239. Kaplan, J.S., Stella, N., Catterall, W.A., and Westenbroek, R.E. (2017). Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. *Proc. Natl. Acad. Sci. USA* 114, 11229–11234.
240. Devinsky, O., Cross, J.H., Laux, L., Marsh, E., Miller, I., Nabbout, R., Scheffer, I.E., Thiele, E.A., and Wright, S.; Cannabidiol in Dravet Syndrome Study Group (2017). Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. *N. Engl. J. Med.* 376, 2011–2020.
241. Chuang, S.H., Westenbroek, R.E., Stella, N., and Catterall, W.A. (2021). Combined antiseizure efficacy of cannabidiol and clobazepam in a conditional mouse model of Dravet syndrome. *J. Exp. Neurol.* 2, 81–85.
242. Martínez-Aguirre, C., Carmona-Cruz, F., Velasco, A.L., Velasco, F., Aguado-Carrillo, G., Cuéllar-Herrera, M., and Rocha, L. (2020). Cannabidiol acts at 5-HT1A receptors in the human brain: relevance for treating temporal lobe epilepsy. *Front. Behav. Neurosci.* 14, 611278.
243. Vitale, R.M., Iannotti, F.A., Schiano Moriello, A., Tunisi, L., Piscitelli, F., Savopoulos, R., Cristino, L., De Petrocellis, L., Amodeo, P., Gray, R., and Marzo, V.D. (2021). Identification and characterization of cannabidiol as an OX1R antagonist by computational and in vitro functional validation. *Biomolecules* 11, 1134.
244. Kathmann, M., Flau, K., Redmer, A., Tränkle, C., and Schlicker, E. (2006). Cannabidiol is an allosteric modulator at μ - and δ -opioid receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 372, 354–361.
245. Rudolph, U., and Knoflach, F. (2011). Beyond classical benzodiazepines: novel therapeutic potential of GABA_A receptor subtypes. *Nat. Rev. Drug Discov.* 10, 685–697.
246. Bakas, T., van Nieuwenhuijzen, P.S., Devenish, S.O., McGregor, I.S., Arnold, J.C., and Chebib, M. (2017). The direct actions of cannabidiol and 2-arachidonoyl glycerol at GABA_A receptors. *Pharmacol. Res.* 119, 358–370.
247. Golovko, T., Min, R., Lozovaya, N., Falconer, C., Yatsenko, N., Tsintsadze, T., Tsintsadze, V., Ledent, C., Harvey, R.J., Belelli, D., et al. (2015). Control of inhibition by the direct action of cannabinoids on GABA_A receptors. *Cereb. Cortex* 25, 2440–2455.
248. Sigel, E., Baur, R., Rácz, I., Marazzi, J., Smart, T.G., Zimmer, A., and Gertsch, J. (2011). The major central endocannabinoid directly acts at GABA(A) receptors. *Proc. Natl. Acad. Sci. USA* 108, 18150–18155. <https://doi.org/10.1073/pnas.1113444108>.
249. Puthenkalam, R., Hieckel, M., Simeone, X., Suwattanasophon, C., Feldbauer, R.V., Ecker, G.F., and Ernst, M. (2016). Structural studies of GABA_A receptor binding sites: which experimental structure tells us what? *Front. Mol. Neurosci.* 9, 44.

250. Zhu, S., Novello, C.M., Teng, J., Walsh, R.M., Kim, J.J., and Hibbs, R.E. (2018). Structure of a human synaptic GABA_A receptor. *Nature* 559, 67–72.
251. Mitchell, S.J., and Silver, R.A. (2003). Shunting inhibition modulates neuronal gain during synaptic excitation. *Neuron* 38, 433–445.
252. Campos, A.C., and Guimarães, F.S. (2009). Evidence for a potential role for TRPV1 receptors in the dorsolateral periaqueductal gray in the attenuation of the anxiolytic effects of cannabinoids. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 1517–1521.
253. Costa, B., Giagnoni, G., Franke, C., Trovato, A.E., and Colleoni, M. (2004). Vanilloid TRPV1 receptor mediates the antihyperalgesic effect of the nonpsychoactive cannabinoid, cannabidiol, in a rat model of acute inflammation. *Br. J. Pharmacol.* 143, 247–250.
254. Iannotti, F.A., Hill, C.L., Leo, A., Alhusaini, A., Soubrane, C., Mazzarella, E., Russo, E., Whalley, B.J., Di Marzo, V., and Stephens, G.J. (2014). Nonpsychoactive plant cannabinoids, cannabidiol (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels in vitro: potential for the treatment of neuronal hyperexcitability. *ACS Chem. Neurosci.* 5, 1131–1141.
255. Mlost, J., Kędziora, M., and Starowicz, K. (2021). Computational approach reveals pronociceptive potential of cannabidiol in osteoarthritis: role of transient receptor potential channels. *Pharmaceuticals (Basel)* 14, 964.
256. Muller, C., and Reggio, P.H. (2020). An analysis of the putative CBD binding site in the ionotropic cannabinoid receptors. *Front. Cell. Neurosci.* 14, 615811.
257. Carrier, E.J., Auchampach, J.A., and Hillard, C.J. (2006). Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc. Natl. Acad. Sci. USA* 103, 7895–7900. <https://doi.org/10.1073/pnas.0511232103>.
258. Hinton, D.J., Lee, M.R., Jang, J.S., and Choi, D.S. (2014). Type 1 equilibrative nucleoside transporter regulates astrocyte-specific glial fibrillary acidic protein expression in the striatum. *Brain Behav.* 4, 903–914.
259. Pastor-Anglada, M., and Pérez-Torras, S. (2018). Who is who in adenosine transport. *Front. Pharmacol.* 9, 627.
260. Rimmerman, N., Ben-Hail, D., Porat, Z., Juknat, A., Kozela, E., Daniels, M.P., Connelly, P.S., Leishman, E., Bradshaw, H.B., Shoshan-Barmatz, V., and Vogel, Z. (2013). Direct modulation of the outer mitochondrial membrane channel, voltage-dependent anion channel 1 (VDAC1) by cannabidiol: a novel mechanism for cannabinoid-induced cell death. *Cell Death Dis.* 4, e949.
261. Huang, C.W., Lin, P.C., Chen, J.L., and Lee, M.J. (2021). Cannabidiol selectively binds to the voltage-gated sodium channel Nav1.4 in its slow-inactivated state and inhibits sodium current. *Biomedicines* 9, 1141.
262. Ghovanloo, M.R., Shuart, N.G., Mezeyova, J., Dean, R.A., Ruben, P.C., and Goodchild, S.J. (2018). Inhibitory effects of cannabidiol on voltage-dependent sodium currents. *J. Biol. Chem.* 293, 16546–16558.
263. Hollister, L.E. (1973). Cannabidiol and cannabiol in man. *Experientia* 29, 825–826.
264. Perez-Reyes, M., Timmons, M.C., Davis, K.H., and Wall, E.M. (1973). A comparison of the pharmacological activity in man of intravenously administered delta9-tetrahydrocannabinol, cannabiol, and cannabidiol. *Experientia* 29, 1368–1369.
265. Devinsky, O., Marsh, E., Friedman, D., Thiele, E., Laux, L., Sullivan, J., Miller, I., Flamini, R., Wilfong, A., Filloux, F., et al. (2016). Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. *Lancet Neurol.* 15, 270–278.
266. Hotz, J., Fehlmann, B., Papassotiropoulos, A., de Quervain, D.J., and Schickel, N.S. (2021). Cannabidiol enhances verbal episodic memory in healthy young participants: A randomized clinical trial. *J. Psychiatr. Res.* 143, 327–333.
267. Bayer, H., Stern, C.A.J., Troyner, F., Gazarini, L., Guimarães, F.S., and Bertoglio, L.J. (2022). Medial prefrontal cortex mechanisms of cannabidiol-induced aversive memory reconsolidation impairments. *Neuropharmacology* 205, 108913. <https://doi.org/10.1016/j.neuropharm.2021.108913>.
268. Espejo-Porras, F., Fernández-Ruiz, J., Pertwee, R.G., Mechoulam, R., and García, C. (2013). Motor effects of the non-psychoactive phytocannabinoid cannabidiol that are mediated by 5-HT_{1A} receptors. *Neuropharmacology* 75, 155–163.
269. Linge, R., Jiménez-Sánchez, L., Campa, L., Pilar-Cuellar, F., Vidal, R., Pazos, A., Adell, A., and Díaz, Á. (2016). Cannabidiol induces rapid-acting antidepressant-like effects and enhances cortical 5-HT/glutamate neurotransmission: role of 5-HT_{1A} receptors. *Neuropharmacology* 103, 16–26.
270. Mottarlini, F., Fumagalli, M., Castillo-Díaz, F., Piazza, S., Targa, G., Sangiovanni, E., Pacchetti, B., Sodergren, M.H., Dell'Agli, M., Fumagalli, F., and Caffino, L. (2022). Single and repeated exposure to cannabidiol differently modulate BDNF expression and signaling in the cortico-striatal brain network. *Biomedicines* 10, 1853.
271. Mandelbaum, D.E. (2016). Cannabidiol in patients with treatment-resistant epilepsy. *Lancet Neurol.* 15, 544–545.
272. Savage, T.E., Sourbron, J., Bruno, P.L., Skirvin, L.A., Wolper, E.S., Anagnos, C.J., and Thiele, E.A. (2020). Efficacy of cannabidiol in subjects with refractory epilepsy relative to concomitant use of clobazam. *Epilepsy Res.* 160, 106263. <https://doi.org/10.1016/j.epilepsyres.2019.106263>.
273. Devinsky, O., Patel, A.D., Cross, J.H., Villanueva, V., Wirrell, E.C., Privitera, M., Greenwood, S.M., Roberts, C., Checketts, D., VanLandingham, K.E., et al. (2018). Effect of cannabidiol on drop seizures in the Lennox-Gastaut syndrome. *N. Engl. J. Med.* 378, 1888–1897.
274. Barker, M.J., Greenwood, K.M., Jackson, M., and Crowe, S.F. (2004). Persistence of cognitive effects after withdrawal from long-term benzodiazepine use: a meta-analysis. *Arch. Clin. Neuropsychol.* 19, 437–454.
275. Chamberlain, J.M., Okada, P., Holsti, M., Mahajan, P., Brown, K.M., Vance, C., Gonzalez, V., Lichenstein, R., Stanley, R., Brousseau, D.C., et al. (2014). Lorazepam vs diazepam for pediatric status epilepticus: a randomized clinical trial. *JAMA* 311, 1652–1660.
276. Jones, N.A., Hill, A.J., Smith, I., Bevan, S.A., Williams, C.M., Whalley, B.J., and Stephens, G.J. (2010). Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. *J. Pharmacol. Exp. Ther.* 332, 569–577.
277. Consroe, P., and Wolkin, A. (1977). Cannabidiol-antiepileptic drug comparisons and interactions in experimentally induced seizures in rats. *J. Pharmacol. Exp. Ther.* 201, 26–32.
278. Patra, P.H., Barker-Haliski, M., White, H.S., Whalley, B.J., Glynn, S., Sandhu, H., Jones, N., Bazelt, M., Williams, C.M., and McNeish, A.J. (2019). Cannabidiol reduces seizures and associated behavioral comorbidities in a range of animal seizure and epilepsy models. *Epilepsia* 60, 303–314.
279. Wright, M., Di Ciano, P., and Brands, B. (2020). Use of cannabidiol for the treatment of anxiety: a short synthesis of pre-clinical and clinical evidence. *Cannabis Cannabinoid Res.* 5, 191–196.
280. Crippa, J.A.S., Derenusson, G.N., Ferrari, T.B., Wichert-Ana, L., Duran, F.L., Martin-Santos, R., Simões, M.V., Bhattacharyya, S., Fusar-Poli, P., Atakan, Z., et al. (2011). Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *J. Psychopharmacol.* 25, 121–130.
281. Bergamaschi, M.M., Queiroz, R.H., Chagas, M.H., de Oliveira, D.C., De Martinis, B.S., Kapczinski, F., Quevedo, J., Roesler, R., Schröder, N., Nardi, A.E., et al. (2011). Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. *Neuropsychopharmacology* 36, 1219–1226.
282. Bloomfield, M.A.P., Yamamori, Y., Hindocha, C., Jones, A.P.M., Yim, J.L.L., Walker, H.R., Statton, B., Wall, M.B., Lees, R.H., Howes, O.D., et al. (2022). The acute effects of cannabidiol on emotional processing and anxiety: a neurocognitive imaging study. *Psychopharmacology* 239, 1539–1549.

283. Shannon, S., and Opila-Lehman, J. (2015). Cannabidiol oil for decreasing addictive use of marijuana: a case report. *Integr. Med. (Encinitas)* 14, 31–35.
284. Shannon, S., Lewis, N., Lee, H., and Hughes, S. (2019). Cannabidiol in anxiety and sleep: a large case series. *Perm. J.* 23, 18–41.
285. Sartim, A.G., Guimarães, F.S., and Joca, S.R.L. (2016). Antidepressant-like effect of cannabidiol injection into the ventral medial prefrontal cortex-possible involvement of 5-HT1A and CB1 receptors. *Behav. Brain Res.* 303, 218–227.
286. Soares, Vde P., Campos, A.C., Bortoli, V.C., Zangrossi, H., Guimarães, F.S., and Zuardi, A.W. (2010). Intra-dorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors. *Behav. Brain Res.* 213, 225–229.
287. Franzen, J.M., Werle, I., Vanz, F., de Oliveira, B.B., Martins Nascimento, L.M., Guimarães, F.S., and Bertoglio, L.J. (2023). Cannabidiol attenuates fear memory expression in female rats via hippocampal 5-HT1A but not CB1 or CB2 receptors. *Neuropharmacology* 223, 109316. <https://doi.org/10.1016/j.neuropharm.2022.109316>.
288. Nielsen, S.W., Hasselsteen, S.D., Dominiak, H.S.H., Labudovic, D., Reiter, L., Dalton, S.O., and Herrstedt, J. (2022). Oral cannabidiol for prevention of acute and transient chemotherapy-induced peripheral neuropathy. *Support. Care Cancer* 30, 9441–9451.
289. van Orten-Luiten, A.-C.B., De Roos, N.M., Majait, S., Witterman, B.J.M., and Witkamp, R.F. (2022). Effects of cannabidiol chewing gum on perceived pain and well-being of irritable bowel syndrome patients: a placebo-controlled crossover exploratory intervention study with symptom-driven dosing. *Cannabis Cannabinoid Res.* 7, 436–444.
290. Silva-Cardoso, G.K., Lazarini-Lopes, W., Hallak, J.E., Crippa, J.A., Zuardi, A.W., Garcia-Cairasco, N., and Leite-Panissi, C.R.A. (2021). Cannabidiol effectively reverses mechanical and thermal allodynia, hyperalgesia, and anxious behaviors in a neuropathic pain model: possible role of CB1 and TRPV1 receptors. *Neuropharmacology* 197, 108712. <https://doi.org/10.1016/j.neuropharm.2021.108712>.
291. Okine, B.N., Mc Laughlin, G., Gaspar, J.C., Harhen, B., Roche, M., and Finn, D.P. (2020). Antinociceptive effects of the GPR55 antagonist CID16020046 injected into the rat anterior cingulate cortex. *Neuroscience* 443, 19–29.
292. Leas, E.C., Hendrickson, E.M., Nobles, A.L., Todd, R., Smith, D.M., Dredze, M., and Ayers, J.W. (2020). Self-reported cannabidiol (CBD) use for conditions with proven therapies. *JAMA Netw. Open* 3, e2020977.
293. Fusar-Poli, L., Cavone, V., Tinacci, S., Concas, I., Petralia, A., Signorelli, M.S., Díaz-Caneja, C.M., and Aguglia, E. (2020). Cannabinoids for people with ASD: a systematic review of published and ongoing studies. *Brain Sci.* 10, 572.
294. Fleury-Teixeira, P., Caixeta, F.V., Ramires da Silva, L.C., Brasil-Neto, J.P., and Malcher-Lopes, R. (2019). Effects of CBD-enriched cannabis sativa extract on autism spectrum disorder symptoms: an observational study of 18 participants undergoing compassionate. *Front. Neurol.* 10, 1145.
295. Pretsch, C.M., Voinescu, B., Mendez, M.A., Wichers, R., Ajram, L., Ivin, G., Heasman, M., Williams, S., Murphy, D.G., Daly, E., and McAlonan, M.G. (2019). The effect of cannabidiol (CBD) on low-frequency activity and functional connectivity in the brain of adults with and without autism spectrum disorder (ASD). *J. Psychopharmacol.* 33, 1141–1148.
296. Hartmann, A., Lisboa, S.F., Sonego, A.B., Coutinho, D., Gomes, F.V., and Guimarães, F.S. (2019). Cannabidiol attenuates aggressive behavior induced by social isolation in mice: involvement of 5-HT1A and CB1 receptors. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 94, 109637. <https://doi.org/10.1016/j.pnpbp.2019.109637>.
297. Hurd, Y.L. (2020). Leading the next CBD wave-safety and efficacy. *JAMA Psychiatry* 77, 341–342.
298. Hurd, Y.L., Spriggs, S., Alishayev, J., Winkel, G., Gurgov, K., Kudrich, C., Oprea, A.M., and Salsitz, E. (2019). Cannabidiol for the reduction of cue-induced craving and anxiety in drug-abstinent individuals with heroin use disorder: a double-blind randomized placebo-controlled trial. *Am. J. Psychiatry* 176, 911–922.
299. Navarrete, F., Gasparyan, A., and Manzaneres, J. (2022). CBD-mediated regulation of heroin withdrawal-induced behavioural and molecular changes in mice. *Addict. Biol.* 27, e13150.
300. Kudrich, C., Hurd, Y.L., Salsitz, E., and Wang, A.L. (2022). Adjunctive management of opioid withdrawal with the nonopioid medication cannabidiol. *Cannabis Cannabinoid Res.* 7, 569–581.
301. Alegre-Zurano, L., Berbegal-Sáez, P., Luján, M.Á., Cantacorps, L., Martín-Sánchez, A., García-Baños, A., and Valverde, O. (2022). Cannabidiol decreases motivation for cocaine in a behavioral economics paradigm but does not prevent incubation of craving in mice. *Biomed. Pharmacother.* 148, 112708.
302. Galaj, E., Bi, G.H., Yang, H.J., and Xi, Z.X. (2020). Cannabidiol attenuates the rewarding effects of cocaine in rats by CB2, 5-HT1A and TRPV1 receptor mechanisms. *Neuropharmacology* 167, 107740. <https://doi.org/10.1016/j.neuropharm.2019.107740>.
303. Luján, M.Á., Alegre-Zurano, L., Martín-Sánchez, A., Cantacorps, L., and Valverde, O. (2022). CB1 receptor antagonist AM4113 reverses the effects of cannabidiol on cue and stress-induced reinstatement of cocaine-seeking behaviour in mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 113, 110462.
304. Sharifi, A., Karimi-Haghighi, S., Shabani, R., Asgari, H.R., Ahadi, R., and Haghighparast, A. (2022). Cannabidiol impairs the rewarding effects of methamphetamine: involvement of dopaminergic receptors in the nucleus accumbens. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 113, 110458.
305. Andaloussi, Z.I.L., Lauer, W., Zulu, S.S., Taghzouti, K., and Abboussi, O. (2021). Acute cannabidiol treatment attenuates ethanol-induced place preference and reduces aggressivity in group-housed male rats. *Pharmacol. biochem. behav.* 211, 173290.
306. Greenwood, L.M., Broyd, S.J., van Hell, H.H., Todd, J., Jones, A., Murray, R.M., Croft, R.J., Michie, P.T., and Solowij, N. (2022). Acute effects of Δ^9 -tetrahydrocannabinol and cannabidiol on auditory mismatch negativity. *Psychopharmacology* 239, 1409–1424.
307. Kaufmann, R., Aqua, K., Lombardo, J., and Lee, M. (2021). Observed impact of long-term consumption of oral cannabidiol on liver function in healthy adults. *Cannabis Cannabinoid Res.* <https://doi.org/10.1089/can.2021.0114>.
308. Kamali, I.G., Shirakawa, I., Kasinski, N., Pfeiffer, A., and Carlini, E.A. (1974). Cannabidiol interferes with the effects of Δ^9 -tetrahydrocannabinol in man. *Eur. J. Pharmacol.* 28, 172–177.
309. Hollister, L.E., and Gillespie, H. (1975). Interactions in man of delta-9-tetrahydrocannabinol. II. Cannabinol and cannabidiol. *Clin. Pharmacol. Ther.* 18, 80–83.
310. Arkell, T.R., Lintzeris, N., Kevin, R.C., Ramaekers, J.G., Vandrey, R., Irwin, C., Haber, P.S., and McGregor, I.S. (2019). Cannabidiol (CBD) content in vaporized cannabis does not prevent tetrahydrocannabinol (THC)-induced impairment of driving and cognition. *Psychopharmacology* 236, 2713–2724.
311. Englund, A., Oliver, D., Chesney, E., Chester, L., Wilson, J., Sovi, S., Micheli, A.D., Hodsoll, J., Poli, P.F., Strang, J., et al. (2022). Does cannabidiol make cannabis safer? A randomised, double-blind, cross-over trial of cannabis with four different CBD: THC ratios. *Neuropsychopharmacology*, 1–8.
312. Salviato, B.Z., Raymundi, A.M., Rodrigues da Silva, T., Saleme, B.W., Batista Sohn, J.M., Araújo, F.S., Guimarães, F.S., Bertoglio, L.J., and Stern, C.A. (2021). Female but not male rats show biphasic effects of low doses of Δ^9 -tetrahydrocannabinol on anxiety: can cannabidiol interfere with these effects? *Neuropharmacology* 196, 108684. <https://doi.org/10.1016/j.neuropharm.2021.108684>.
313. Winstone, J., Shafique, H., Clemmer, M.E., Mackie, K., and Wager-Miller, J. (2022). Effects of tetrahydrocannabinol and cannabidiol on brain-derived neurotrophic factor and tropomyosin receptor kinase B expression in the adolescent Hippocampus cannabis and cannabinoid research. <https://doi.org/10.1089/can.2021.0025>.
314. Taffe, M.A., Creehan, K.M., and Vandewater, S.A. (2015). Cannabidiol fails to reverse hypothermia or locomotor suppression induced by Δ^9

- tetrahydrocannabinol in Sprague-Dawley rats. *Br. J. Pharmacol.* **172**, 1783–1791.
315. Gabaglio, M., Zamberletti, E., Manenti, C., Parolaro, D., and Rubino, T. (2021). Long-term consequences of adolescent exposure to THC-rich/CBD-poor and CBD-rich/THC-poor combinations: A comparison with pure THC treatment in female rats. *Int. J. Mol. Sci.* **22**, 8899.
316. Cuttler, C., LaFrance, E.M., and Stueber, A. (2021). Acute effects of high-potency cannabis flower and cannabis concentrates on everyday life memory and decision making. *Sci. Rep.* **11**, 13784.
317. Zelasko, S., Arnold, W.R., and Das, A. (2015). Endocannabinoid metabolism by cytochrome P450 monooxygenases. *Prostaglandins Other Lipid Mediat.* **116–117**, 112–123.
318. Atwood, B.K., Huffman, J., Straiker, A., and Mackie, K. (2010). JWH018, a common constituent of 'Spice' herbal blends, is a potent and efficacious cannabinoid CB1 receptor agonist. *Br. J. Pharmacol.* **160**, 585–593.
319. Sexton, M., Shelton, K., Haley, P., and West, M. (2018). Evaluation of cannabinoid and terpenoid content: cannabis flower compared to supercritical CO2 concentrate. *Planta Med.* **84**, 234–241.
320. Santos, A.G., da Rocha, G.O., and de Andrade, J.B. (2019). A novel phytocannabinoid isolated from *Cannabis sativa* L. with an in vivo cannabinomimetic activity higher than Δ^9 -tetrahydrocannabinol: Δ^9 -Tetrahydrocannabiphorol. *Sci. Rep.* **9**, 1.
321. Dvorakova, M., Wilson, S., Corey, W., Billingsley, J., Zimmowitch, A., Tracey, J., Straiker, A., and Mackie, K. (2022). A critical evaluation of terpenoid signaling at cannabinoid CB1 receptors in a neuronal model. *Molecules* **27**, 5655.
322. Tagen, M., and Klumpers, L.E. (2022). Review of delta-8-tetrahydrocannabinol (Δ^8 -THC): comparative pharmacology with Δ^9 -THC. *Br. J. Pharmacol.* **179**, 3915–3933.
323. Thapa, D., Cairns, E.A., Szczesniak, A.M., Toguri, J.T., Caldwell, M.D., and Kelly, M.E.M. (2018). The cannabinoids Δ^8 THC, CBD, and HU-308 act via distinct receptors to reduce corneal pain and inflammation. *Cannabis Cannabinoid Res.* **3**, 11–20.
324. Terry, G., Liow, J.S., Chernet, E., Zoghbi, S.S., Phebus, L., Felder, C.C., Tauscher, J., Schaus, J.M., Pike, V.W., Halldin, C., and Innis, R.B. (2008). Positron emission tomography imaging using an inverse agonist radioligand to assess cannabinoid CB1 receptors in rodents. *Neuroimage* **41**, 690–698.
325. Hirvonen, J., Zanotti-Fregonara, P., Gorelick, D.A., Lyoo, C.H., Rallis-Frutos, D., Morse, C., Zoghbi, S.S., Pike, V.W., Volkow, N.D., Huestis, M.A., and Innis, R.B. (2018). Decreased cannabinoid CB1 receptors in male tobacco smokers examined with positron emission tomography. *Biol. Psychiatry* **84**, 715–721.
326. Cao, S., Kang, S., Mao, H., Yao, J., Gu, L., and Zheng, N. (2022). Defining molecular glues with a dual-nanobody cannabidiol sensor. *Nat. Commun.* **13**, 815.
327. Dong, A., He, K., Dudok, B., Farrell, J.S., Guan, W., Liput, D.J., Puhl, H.L., Cai, R., Wang, H., Duan, J., et al. (2022). A fluorescent sensor for spatiotemporally resolved imaging of endocannabinoid dynamics in vivo. *Nat. Biotechnol.* **40**, 787–798.
328. Day, A.L., Greisen, P., Doyle, L., Schena, A., Stella, N., Johnsson, K., Baker, D., and Stoddard, B. (2018). Unintended specificity of an engineered ligand-binding protein facilitated by unpredicted plasticity of the protein fold. *Protein Eng. Des. Sel.* **31**, 375–387.