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Review Article

Neurotoxic or Protective Cannabis Components: Delta-9-Tetrahydrocannabinol ($^{\Delta 9}$THC) and Cannabidiol (CBD)

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Abstract

Cannabis sativa contains phytocannabinoids that are psychoactive and neurotoxic (delta-9-tetrahydrocannabinol: $^{\Delta 9}$THC) or nonpsychoactive and presumptively neuroprotective (cannabidiol: CBD). Along with rising legalization, availability, and demand, the $^{\Delta 9}$THC:CBD ratio also has increased. Cannabis legalization means that use will likely increase in pregnant or breastfeeding women, affecting all stages of brain and neurodevelopment of their offspring. $^{\Delta 9}$THC exposure *in utero* or during development leads to lasting detrimental effects on behavior, cognition, locomotor activity, as well as epigenetic changes. Caution is urged with cannabis use. CBD is one of the most actively studied therapies for a broad spectrum of neurological, inflammatory, and mental diseases (e.g., Parkinson's disease, Huntington's disease, Alzheimer's disease, schizophrenia) because of its efficacy, low toxicity, and availability. While data indicate that the benefits of CBD may outweigh its risks, there are indications that it poses a risk for adverse effects on neurodevelopment from *in-utero* exposure as well as detrimental effects on male reproduction. Therefore, there is a clear need to continue researching the effects of $^{\Delta 9}$THC exposure as well as the optimal CBD treatment related to disease management while stressing the need to further characterize possible adverse effects.

Introduction

Medicinal and recreational cannabis use has increased globally, and continuation of this trend is anticipated as its use becomes legalized internationally.[1,2] *Cannabis sativa* is composed of over 100 "cannabinoids,"[3,4] but the psychoactive compound delta-9-tetrahydrocannabinol ($^{\Delta 9}$-THC), isolated in 1964, and the nonpsychoactive compound cannabidiol (CBD), isolated in 1940,[5] represent the most abundant components. Consumption of cannabis products occurs through diverse routes (inhaled smoke, vaping of liquid extracts, resins or waxes, lotions, edibles).[6,7] Inhaled cannabinoids are rapidly absorbed in the lungs[8] but less so by other routes (e.g., dermal, oral, rectal).[9] Due to their highly lipophilic properties, they are stored in adipose tissue for weeks or months and are concentrated in the breast milk of rodents and humans.[10,11] CBD products can have beneficial health effects and aid in various medical disorders (e.g., Parkinson's disease, anxiety, and epilepsy).[12,13] Accumulating evidence also indicates there are neurotoxic and reproductive effects from exposure.[14–18]

Due to increasing cannabis use, exposure to $^{\Delta 9}$-THC presents concerning health risks because use will likely also increase in pregnant or breastfeeding women, affecting all stages of brain and neurodevelopment of their offspring.[19–24] Along with increased legalization, social acceptance, and use, a change in the ratio of $^{\Delta 9}$-THC to CBD in cannabis has also occurred, leading to a change in potency (the $^{\Delta 9}$-THC:CBD ratio increased from 14:1 in 1995 to 80:1 in 2014).[25] Ultimately, the extent of cannabis neurotoxicity[26] is dependent on many variables, including the $^{\Delta 9}$-THC exposure level, purity,[25] route of administration,[7,9,27] developmental age at exposure,[23,28–30] health status,[31,32] pregnancy status,[21,33–36] lactational status,[37,38] and others.[39] Further, due to the lipophilic nature of these compounds, it has been shown that exposure at low, re-

Keywords: $^{\Delta 9}$THC, Delta-9-Tetrahydrocannabinol; Cannabidiol; Neurodevelopment; Endocannabinoid system; Cannabis; Neuroprotection; Neurotoxicity.

Abbreviations: ACh, acetylcholine; 2-AG, 2-arachidonoylglycerol; 5-HT, serotonin; AEA, anandamide; ARfD, acute reference dose; βA, beta-amyloid; BDNF, brain-derived neurotropic factor; Ca^{+2}, calcium; CB1R, cannabinoid 1 receptor; CBD, cannabidiol; CNS, central nervous system; COX2, cyclooxygenase-2; D1 or D2, dopamine receptors; DA, dopamine; DAGL, diacylglycerol lipase; DRN, dorsal raphe nucleus; DS, Dravet syndrome; eCB, endocannabinoid; eCBS, endocannabinoid system; FAAH, fatty acid amide hydrolase; GABA, gamma-aminobutyric acid; GD, gestation day; GPR55, G-coupled protein receptor 55; i.p., intraperitoneal; i.v., intravenous; IL, interleukin; iNOS, inducible nitric oxide synthase; K^+, potassium; LOAEL, lowest-observed-adverse-effect level; LOEL, lowest-observed-effect level; MAGL, monoacylglycerol lipase; MOA, mode of action; NAc, nucleus accumbens; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; NMDA, *N*-methyl-D-aspartate; NOAEL, no-observed-adverse-effect level; PFC, prefrontal cortex; PPARγ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; s.c., subcutaneous; SNc, substantia nigra; TNF, tumor necrosis factor; TRPV1, transient receptor potential cation channel subfamily V member 1, or vanilloid receptor 1; VTA, ventral tegmental area; $^{\Delta 9}$THC, delta-9-tetrahydrocannabinol.

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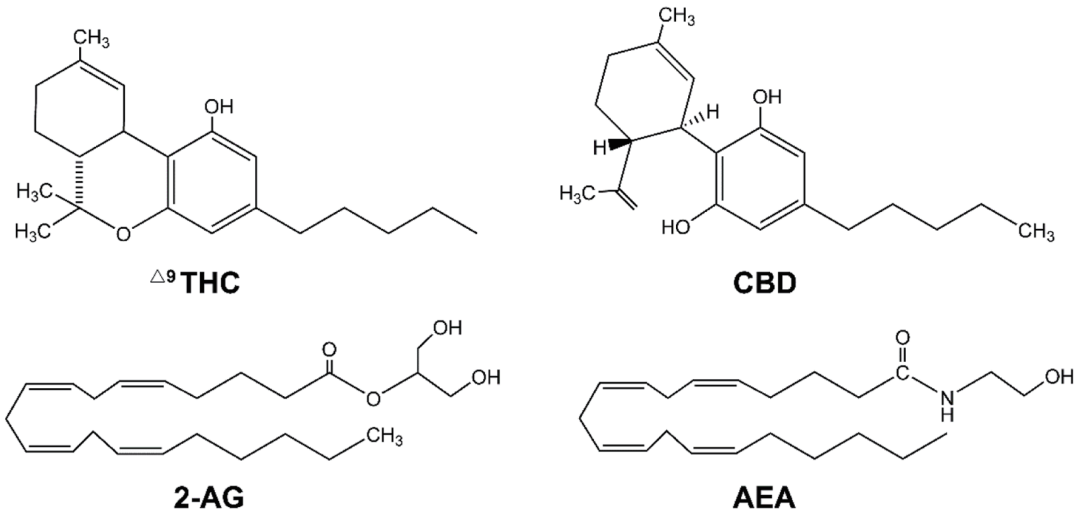

Fig. 1. Lipophilic structures for delta-9-tetrahydrocannabinol ($^{\Delta 9}$THC) and cannabidiol (CBD) as well as the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA). Each compound acts at the G protein-coupled receptors cannabinoid 1 and 2 receptors, which affect neurotransmitter release.

alistically achievable *in-vivo* concentrations causes specific molecular targets to be affected, resulting in behavioral or cognitive deficits in those with $^{\Delta 9}$THC exposure,[39–41] or potential benefits that greatly improve the health of those with neurodegenerative diseases.[42–44]

In this review, both the risks and benefits of exposure associated with $^{\Delta 9}$THC and CBD were investigated. Notably, the risks from CBD exposure, which is usually considered to be safe, are associated with reproductive and developmental health effects.[45] Recently, concerns have been raised about CBD use, since it is available in numerous over-the-counter products, with little data supporting its safety or efficacy.[46] The side effects and adverse health effects, along with questions regarding the ingredients, are often unknown. On the other hand, $^{\Delta 9}$THC exposure has been associated with adverse effects, depending on the dose, yet the benefits of this drug need to be emphasized. These phytocannabinoids were selected because they are the dominant compounds in cannabis, and they are often used as treatments for physical ailments as well as for recreational use. There is a vast amount of literature characterizing these compounds and their effects during development and throughout life in both animal and human studies, but it is important to present the risks as well as the benefits.

The endocannabinoid system (eCBS)

The eCBS was discovered in the 1990s while investigating the mode of action (MOA) of $^{\Delta 9}$THC. It is innate and multifaceted, affecting metabolic pathways throughout the body [e.g., muscle, adipose tissue, gastrointestinal tract, liver, and central nervous system (CNS)].[47] It helps to shape neuronal connectivity in the brain throughout development and into adulthood,[48] affecting the gamma-aminobutyric acid (GABA)ergic, glutamatergic, opioid, and dopaminergic systems.[49] Cell membrane-bound cannabinoid-1 receptors (CB1Rs) are the most abundant in the brain, while CB2Rs are mainly expressed on immune cells (T-cells, macrophages) in the periphery or glia/microglia in the brain.[47,50] Some researchers have suggested that the transient receptor potential cation channel subfamily V member 1 (TRPV1 or vanilloid receptor 1) could be classified as CB3R, as it is activated by CBD.[51] Each receptor type can act independently; however, depending on their location, CB1Rs and CB2Rs (possibly also CB3Rs) can act together, competitively, or in opposite directions, potentially through dimerization to regulate physiological effects.

Normally, neurotransmitters [e.g., glutamate, GABA, serotonin (5-HT), dopamine (DA), acetylcholine (ACh), or norepinephrine] in the CNS are released presynaptically via neuronal stimulation, or by G protein-coupled receptors and voltage-gated ion channel calcium (Ca^{+2}) and potassium (K^{+}) influx.[50,52] However, the elevation in postsynaptic Ca^{+2} affected by neurotransmitters/receptors through the ion channels [e.g., ionotropic glutamate receptors, *N*-methyl-D-aspartate (NMDA), or GABA],[53] stimulates endocannabinoid (eCB) postsynaptic biosynthesis.[50,52,54]

There are two principal eCB ligands [2-arachidonoylglycerol (2-AG) and anandamide (AEA)], which are synthesized postsynaptically from arachidonic acid by *N*-acyl phosphatidylethanolamine phospholipase D and diacylglycerol lipase alpha/beta (DAGLα/β), respectively.[55–57] These eCBs are produced, as needed,[47] postsynaptically by Ca^{+2}-dependent transacyclase and other enzymes, then they migrate from postsynaptic neurons to the presynaptic CBR.[53,58] Signaling then occurs as CBR couples to the guanosine-5′-triphosphate ($G_{i/o}$)/α-protein subunit dimer[58,59] and binds adenyl cyclase to generate cyclic adenosine monophosphate. The cascade decreases presynaptic Ca^{+2} influx by blocking the activity of voltage-dependent N-, P/Q- and L-type Ca^{+2} channels[60,61] and activation of some K^{+} channels.[53,62] The retrograde eCB (AEA and 2-AG) transmitters in the brain presynaptically inhibit the release of the neurotransmitters GABA,[63,64] glutamate,[63,65,66] DA,[65,67,68] norepinephrine,[69] 5-HT[67,70] and ACh,[71,72] thereby decreasing the probability of neurotransmitter release. eCBs are then degraded by the serine hydrolase monoacylglycerol lipase (MAGL) in the presynaptic cell and fatty acid amide hydrolase (FAAH) located in the postsynaptic cell.[49,57,73]

Figure 1 compares the lipophilic structures of the eCBs (2-AG and AEA) with cannabinoids (e.g., $^{\Delta 9}$THC and CBD). $^{\Delta 9}$THC and CBD toxicity or neuroprotection depends on factors such as potency, exposure, duration/frequency, vehicle, route of administration, and species-specific differences. Pharmacokinetic and pharmacodynamic parameters determine the extent of P450 (CYP1A, 3A4, 2C9, and 2C19) metabolic activation and glucuronidation elimina-

Table 1. Brain regions and pathways affected by endocannabinoids, $^{\Delta 9}$-THC and/or CBD

Neurotransmitter/Pathway	Brain region associations	Behavior/processes involving eCBS	Reference
Dopamine: DA			
Mesolimbic	DA from ventral tegmental area (VTA; midbrain) → ventral striatum (amygdala, pyriform cortex, lateral septal nuclei, nucleus accumbens)	Reward-related cognition (e.g., incentive: wanting; pleasure: liking; positive reinforcement, associative learning) & emotion	78,80,81,88–91
Mesocortical	DA from VTA (midbrain) → prefrontal cortex + hippocampus	Cognition: executive function (e.g., planning, attention, working memory, planning, self-control, etc.), emotion	
Nigrostriatal	DA from substantia nigra (pars compacta; substantia nigra SNc: midbrain) → dorsal striatum (i.e., caudate nucleus + putamen)	Neuromotor function, reward-related cognition, associative learning	
Tuberoinfundibular	DA from the hypothalamic arcuate (infundibular) + paraventricular nucleus → pituitary gland median eminence	Inhibits the release of prolactin.	
Glutamate			
Glutamatergic	Hippocampus, neocortex and over 90% of synapses in human brain.	Excitatory effects on VTA & SNc neurons, memory, learning, neural communication	53,90,92,93
γ-Aminobutyric Acid: GABA			
GABAergic	Hippocampus, thalamus, basal ganglia, hypothalamus, brainstem[a]	Inhibitory effects on VTA and SNc neurons	90,94–96
Serotonin: 5HT			
Serotonergic	Dorsal raphe nuclei, cortex, hippocampus	Modulator of receptors with effects depending on subtype (i.e., biphasic effect on VTA neurons)	80,84,85,97,98

[a]GABAergic transmission includes inhibitory median spiny neurons in the striatum/basal ganglia affected by the glutamatergic (AMPAR) and dopaminergic (D1 and D2) receptor inputs from the VTA, SNc, and PFC.[87]

tion of $^{\Delta 9}$THC and CBD.[9,74] A tipping point leading to an adverse health effect would depend on an individual's ability to handle various exposure loads based on age, genetic makeup, health status, and diet, among other influences.[75,76] These risk factors are often difficult to characterize in humans, since hepatic metabolism studies are, by necessity, generally performed *in vitro*.[75]

$^{\Delta 9}$THC-associated mechanisms and neurotoxicity

To understand the effects of $^{\Delta 9}$THC on the brain, it is helpful to know which areas are affected. The eCBS/CBRs throughout the brain[77] help to regulate glutamatergic (excitatory), GABAergic (inhibitory),[78,79] dopaminergic, and serotonergic neurotransmitter release at presynaptic terminals.[80,81] The interactions among these systems are complex, occurring via direct and indirect stimulation, which may or may not be overseen by the eCBS to regulate neuroplasticity and excitability toward locomotor activity, cognition (learning and memory), executive functions, reward, motivation, and neuroendocrine control, among other functions.[78,80,82–86] The striatum in the basal ganglia contains inhibitory GABAergic medium spiny neurons that are affected by the glutamatergic (AMPAR) and dopaminergic (i.e., D1 and D2) receptor inputs from the ventral tegmental area (VTA), substantia nigra (SNc), and prefrontal cortex (PFC).[87]

Table 1 summarizes some of the main brain regions, pathways, and neurotransmitters involving the neuronal connections in the eCBS and affected by $^{\Delta 9}$THC.[53,78,80,81,84,85,87–98]

Cannabinoid signaling can be disrupted through agonistic activity of $^{\Delta 9}$THC at the CB1Rs throughout areas of the brain. This process leads to inhibition of accumulation of 2-AG and AEA in the brain.[73,99,100] While there are many other neuronal circuits associated with the eCBS, the ones mentioned above are most frequently associated with cannabis.

$^{\Delta 9}$THC-associated neurotoxicity in rodent and nonhuman primate models

$^{\Delta 9}$THC exposure throughout all life stages is associated with effects on behavior, cognition, locomotor activity, birth weight, learning, and other adverse effects.[101–104] Cannabis smoke was listed as a reproductive toxicant on 3 January 2020, under California's Proposition 65.[104] However, to control for the dose intake and other technical issues, many neurodevelopmental studies performed in animals used intravenous (i.v.) $^{\Delta 9}$THC administration. Although this is not a likely exposure scenario for humans, the immediate absorption by i.v. could be compared to pulmonary exposure by inhalation.[105,106] Subcutaneous (s.c.), oral (i.e., gavage), and intraperitoneal (i.p.) administration are more slowly absorbed and are subject to local metabolic processes prior to entering the blood stream.[107,108] Other considerations contributing to potential variabilities in evaluating the study results are as follows: 1) often only a single exposure dose was used, limiting potential observations of a dose–response relationship; 2) $^{\Delta 9}$THC dosing vehicles varied among studies; 3) different species/strains of rodent were

used; 4) different exposure scenarios were used; and 5) many different laboratories contributed to the list of studies.

Gestational exposure to $^{\Delta 9}$THC

The eCBS is involved in the earliest developmental stages, including fertilization, implantation, and neuronal progenitors in the brain, leading to migration, morphogenesis, and axonal guidance.[94,109,110] The effects of $^{\Delta 9}$THC on these processes can be seen in rodents' pulmonary exposure by inhalation.[105,106] Administration via a s.c., oral, or i.p. route is more slowly absorbed and is subject to local metabolic processes prior to entering the blood stream.[107,111] $^{\Delta 9}$THC has profound effects on CB1Rs in areas of the brain regulating GABA, 5-HT, glutamate neurotransmitters, and DA release, influencing, for example, the development of locomotor activity, cognition, learning, memory, and emotional regulation (Table 2).[11,29,34,112–141] Notably, the lowest doses of $^{\Delta 9}$THC (0.15 mg/kg/day) in the offspring of Long-Evans rats treated *in utero* affected preproenkephalin, an endogenous opioid precursor in the nucleus accumbens, amygdala, and striatum, in addition to showing evidence of decreased cognition and other behavioral effects.[112–114] Treatment *in utero* or from paternal exposure during a full cycle of sperm development, even at low $^{\Delta 9}$THC doses (0.15 mg/kg/day), resulted in developmental deficits and epigenetic transmission.[112,113,115–117] Male Wistar adult rats treated throughout sperm development (gavage, 2.0 mg/kg/day) had offspring with affected locomotor activity, feeding behavior, and visual operant signaling.[118] Moreover, epidemiological evidence supported findings that cannabis exposure during gestation or during male sperm development results in children with cognitive, motor, and behavioral (including severe psychoses) effects.[33,142–145] Infants with gestational exposure to cannabis may show an exaggerated startle response or an inability to adapt to novel stimuli.[146,147] Furthermore, women who used cannabis during pregnancy had an increase in fetal deaths, premature births, heart rhythm disorders, and fetal intrauterine growth restrictions.[36]

In support of the gestational exposure findings, a meta-analysis was performed on the behavioral effects in animal offspring exposed to $^{\Delta 9}$THC during gestation and lactation.[148] A compilation and meta-analysis of behavior in offspring from 15 selected studies in Long-Evans, Sprague-Dawley, or Wistar female and/or male rats exposed from mothers exposed via oral, i.v., or s.c. administration indicated significant effects on cognitive, locomotor, and emotional behavior.

Postnatal exposure to $^{\Delta 9}$THC

Postnatal exposures to young C57BL/6J male mouse pups resulted in behavioral effects from $^{\Delta 9}$THC treatment at 1.0 mg/kg/day administered s.c.[119] This and other studies performed in male and female Wistar rats at 2.0 mg/kg/day (s.c.)[11] or 10 mg/kg/day (gavage)[149] included effects on anxiety and neurodevelopmental deficits similar to those seen in autism, epilepsy, and schizophrenia.[11,119,149] Perinatal exposure in children would likely be from nursing, secondhand smoke, or accidental ingestion, causing long-term effects.[37,150] The transfer of cannabis in the milk to nursing babies was shown to affect DA receptors, resulting in hyperactivity, poor coordination, and cognitive function and leading to an increased risk of future drug abuse.[37,150] For example, GABA is primarily excitatory in early development and then it switches to inhibitory postnatally. Disruption of this process in humans may result in neurodevelopmental patterns affecting chronic pain, neuroplasticity, and psychiatric diseases (e.g., autism, epilepsy, and schizophrenia).[11,151] Data indicate that perinatal cannabis exposure increases the risk of future drug use.[152]

Adolescent exposure to $^{\Delta 9}$THC

Exposure to $^{\Delta 9}$THC i.p. in Long-Evans and Wistar male rats throughout adolescence at low doses (1 or 1.5 mg/kg/day) showed disrupted neural development in the PFC and hippocampus resulting in effects on neuroplasticity, cognition, social interactions, memory and others.[29,120] Similar effects (i.e., increased: CB1R density, anxiety, learning deficits, anhedonia) were observed at higher doses (2.5–10 mg/kg/day) in Long-Evans females, male and female Sprague-Dawley rats and male CD1 mice receiving various dosing regimens (Table 2).[120–123,153] $^{\Delta 9}$THC treatment in adolescents disrupted development of brain areas (e.g., PFC) associated with adverse behaviors like schizophrenia in humans, which often occurs in adolescence.[155] Adolescence is a stage of peak eCB (2AG and AEA) and CB1R expression.[156] The brain is still developing and is at heightened risk for disruption of normal neurodevelopmental processes.[157,158] Where pre- or postnatal exposures may be involuntary in developing young, adolescence is where preteens and teens may begin to experiment with cannabis on their own.[159] Vaping cannabis has become one of the most preferred methods of consumption, that will not only increase the concentration of $^{\Delta 9}$THC but also potentially increase exposure to residues of pesticides used on cannabis crops.[99,160–162] Cannabis use in adolescents greatly increases the risk of psychosis by 3–4-fold and has been shown to lower the age of schizophrenia onset.[163,164] Further, adolescent cannabis use will increase the probability of future drug use,[165] as shown by evidence from animal and epidemiological studies.[152,166,167]

Adult exposure to $^{\Delta 9}$THC

Acute adult effects in Long-Evans male rats as well as C57BL/6Arc and CD1 male mice showed behavioral effects (attention and learning, decreased anxiety and locomotor activity) at low $^{\Delta 9}$THC doses (i.p.: 0.25, 0.8, or 1.0 mg/kg/day; Table 2).[124–127] Notably, these studies used 2–8 treatment levels and could therefore establish a dose–response relationship. C57BL/6J male mice treated at 10 mg/kg/day also experienced a decreased thermic response and increased catalepsy and analgesia.[126] This study demonstrated the "cannabinoid tetrad": increased catalepsy, hypomobility, hypothermia, and antinociception.[128] At the low acute doses, the animals showed decreased anxiety; but at higher doses graduating from 1 to 3 to 10 mg/kg/day at 7-day intervals, the animals had increased anxiety measures with both acute and chronic exposures in male Wistar rats.[129] Human studies also showed that cannabis use versus nonuse was associated with an earlier onset of psychoses, death by suicide, depression, mania, anhedonia, cognitive deficits, and anxiety/paranoia as well as brain effects (decreases in glutamine, affected DA, and decreased hippocampal volume (systematic review).[168] This review also reported associated harmful effects of exposure on driving, stroke, pulmonary function, vision, and negative drug-drug interactions. With cannabis legalization, it is likely that there will be more health-related deficits and an increased need for public and clinical policy changes. Table 2 lists the lowest-observed-effect levels (LOELs) reported from each *in-vivo* study (mg/kg/day).

Nonhuman primate exposure to $^{\Delta 9}$THC

Studies in nonhuman primates have been performed in pregnant animals. Rhesus macaques were fed $^{\Delta 9}$THC in a cookie at 2.5 g/7 kg at gestation day (GD) 0–155.[169] There were decreases in the

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Table 2. Neurotoxic and behavioral effects from $^{\Delta 9}$THC treatment during development in animal studies

Animal strain/Sex/Duration/Dose/Vehicle	Day tested	Effects	LOEL (mg/kg/day)	Reference
		$^{\Delta 9}$THC in animal studies		
Gestational treatment				
Long-Evans Dam: GD 5-PND 2; F1 fostered PND 2–21. Dose: i.v. 0.15 mg/kg/day. Vehicle: Tween 80/saline	F1 M/F Pups: PND 2 or PND 62, Adult	NAc: ↓striatal DRD2 mRNA expression; ↓DR2 receptor & binding sites; epigenetic regulation of DRD2 mRNA expression disrupted; affected DA receptor gene regulation. Significance: Increase in sensitivity to opiate reward in adulthood	0.15*	112
Long-Evans Dam: GD 5-PND 2 fostered PND 2-21. Dose: i.v. 0.15 mg/kg/day. Vehicle: Tween 80/saline	F1 M Pups PND 55, Adult	↓PENK mRNA expression NAc (pup), ↑PENK in NAc & amygdala (adults); ↑Self-administer heroin; ↓latency between active lever press; ↑active lever press; ↑responses on stress test; ↑total responses on active lever on 1st & last extinction days; ↓distance traveled during acquisition & maintenance. Significance: Increased opioid seeking behavior (motivation/reward) & stress response in adulthood	0.15*	114
Long-Evans Dam every 3rd day; PND 28–49; mated PND 64–68; F1 fostered. Dose: i.p. 1.5 mg/kg/day. Vehicle: saline/Tween 80	F1 M/F Pups: PND 35 (Adolescence) or PND 62 Adult	Striatal dysregulation of CB1R gene expression, affecting striatal plasticity; ventral to dorsal striatum disruptions between adolescence & adulthood; F ↓novelty seeking. Significance: Supports relevance to age-dependent vulnerability for neuropsychiatric disorders	1.5*	130
Long-Evans Dam every 3rd day PND 28–49; mated PND 64–68; F1 fostered. Dose: i.p. 1.5 mg/kg/day. Vehicle: saline/Tween 80	F1 M/F Pups: PND 35 (Adolescence) or PND 62, Adult	Epigenetic effects & altered CB1R mRNA expressions in NAc associated with glutamatergic system regulation; F ↓ locomotor activity. Significance: Cross-generational epigenetic vulnerability to drug abuse	1.5*	117
Wistar Dam: GD 15–PND 9. Dose: Gavage 3.0 mg/kg/day. Vehicle: sesame oil	F1 M Pup: PND 90, Adult	Disrupted hippocampal GABAergic system; ↓GABA outflow & uptake in hippocampus; ↓ CB1 binding; cognitive impairments. Significance: Long term cognitive deficiency & disrupted GABA neuronal development	5.0*	115
Wistar Dam: GD 5–14, 16, 18, 21 & PND 1 & 5. Dose: Gavage 5.0 mg/kg/day. Vehicle sesame oil	F1 M/F GD 14, 16, 18, 21 + PND 1 & 5 Neonate	Disrupted tyrosine hydroxylase gene activation (rate limiting in DA production); ↑ DOPACL DA metabolite forebrain. Significance: Tyrosine hydroxylase plays a large part in neurodevelopment through DA production	5.0*	131
Wistar Dam: GD 7–22. Dose: i.p. 3 mg/kg/day. Vehicle: Not stated	F1 M/F Behavior PND 70–100	M: ↓Time on light side of test box (↑anxiety); ↑transition to light; ↓Time in open arm of EPM; ↑VTA spike activity; ↓DA & NMDAR2B PND 21; ↑GAD87 PND 21; F: ↑GAD67, vGLUT1-2; PPARα & PPARY1-2 & NMDAR2B in the mesolimbic system (VTA-NAc); M/F: ↑Altered fatty acid concentrations in the nucleus accumbens core & shell up to PND 120 (M) or PND 21 (F). Significance: Sex difference with M more affected than F; Fatty acid deficits disrupt the DA/GLUT/GABAergic neurotransmissions affecting neurodevelopment	3.0*	132
SD Dam: GD 5–PND 2 foster-nursed PND 2–21. Dose: i.v. 0.15 mg/kg/day. Vehicle: Tween80/saline	F1 M/F Pups: PND 22, 45 & 60 Weaning, adolescent, adult	Pup: ↓anxiety: ↓active place avoidance acquisition; ↑active place avoidance reversal phase entries; Adult: ↓attention (acquisition, reversal & distraction) & cognition. Significance: Decreased anxiety, attention & cognitive function	0.15*	113

(continued)

Table 2. *(continued)*

Animal strain/Sex/Duration/Dose/Vehicle	Day tested	Effects	LOEL (mg/kg/day)	Reference
		$^{\Delta 9}$THC in animal studies		
SD Dam: Group 1: GD 5–20. Group 2: GD 5–20 + PND 15. Dose: s.c. 2.0 mg/kg/day; PND 15 2.5 mg/kg/day. Vehicle: Tween80/saline	F F1 Pups: Groups 1 & 2: PND 15–28 Juvenile	Group 1 & 2: Male behaviors affected: ↑distance traveled; ↓stretch-attend postures; Group 2: ↓ latency in passive avoidance training; ↑AMPA from DA cells; ↓stretch-attend postures; ↓DA 240 min postacute dose. Significance: Behavioral effects from mesolimbic (NAc) dopaminergic disruptions are greater in males & greater after $^{\Delta 9}$THC challenge	2.0*	1331
SD Dam: GD 5–GD 20. Dose: s.c. 2.0 mg/kg/day. Vehicle Tween 80/saline	F1 M/F Pups: Tests done PND 24–28, Juvenile	VTA DA neuron effects: ↑ firing rate; ↓ cells/track; ↓ spikes/burst, burst rate; ↓after hyperpolarization period; ↑DRD2 sensitivity & acute stress vulnerability; ↑ activity, ↓ PPI average in acute restraint & forced swim test. Significance: Sensorimotor gating deficits leading to an increase in susceptivity to stimuli triggering psychotic-like behaviors	2.0*	134
SD M Adult 28 days; mated 2 days post dose. Dose: s.c. 2.0, 4.0 mg/kg/day. Vehicle: Tween 80/saline	F1 M Pups: PND 30, 60, 100 & 150 Adolescent, adult	↓ACh activity; ↑ ChAT: ACh biomarker for number of ACh terminals in striatum; ↓ ChAT hippocampus; ↓HC3/ChAT (ACh activity index) n frontal/parietal cortex & striatum. Significance: Paternal $^{\Delta 9}$THC leads to disruptions in developmental trajectory of ACh potentially affecting attention	2.0	116
Wild-type Mouse Dam: GD 12.5–16.5. Dose: i.p. 3.0 mg/kg/day. Vehicle: saline/DMSO/Tween 80	F1 M/F Pups: PND 20; 2 months; Juvenile, adult	CB1R →affected cortical neuron synaptic signaling development →affected connectivity in cortical GABAergic & glutamatergic systems → ↓fine motor skills; ↓ skilled motor function; 2 months: ↓ success in pellet retrieved in skilled steps test; ↑seizure. Significance: Disrupted CB1 signaling leading to disrupted glutamate & GABA signaling leads to increased susceptibility to seizures and cortico-spinal function.in adulthood	3.0*	135
C57Bl/6 Mouse Dam: GD 14.5–18.5. Dose: i.p. 3.0 mg/kg/day. Vehicle: DMSO	F1 M/F: GD 18.5; PND 10 & 120, Fetal, pup, adult	↓ CB1R & misrouted hippocampal CB1R afferents, ↑CB1R density in striatum; Impaired LTD in pyramidal cell synapsis; ↓ synaptic plasticity in the cortical circuitry; Impaired cortical axonal development; ↓2-AG signaling, ↓ CB1R & ↑ MAGL expression, ↓DAGL; abnormal growth cones & cytoskeleton in axonal region. Significance: Abnormal axonal development in growth cone disrupts neuronal circuitry, memory encoding, cognition & executive skills	3.0*	136
Postnatal Treatment				
Wistar Dam: PND 1–10. Dose: s.c.2.0 mg/kg/day. Vehicle: DMSO/cremophore/saline	F1 M/F Pups: PND 10, 15, 20; 9–21 Preweaning, juvenile	↓Bodyweight gain; GABA excitatory to inhibitory switch in PFC (eCB disruption); ↓upregulation & expression of KCC2 (K+ transporter), Vocalizations ↑in frequency (kHZ). Significance: Delayed development of GABA switch leads to sensorimotor gating deficits, potential autism, epilepsies, schizophrenia-like behavior.	2*	11
Wistar M Adult: 12 days mated to untreated F. Dose: Gavage 2.0 mg/kg/day. Vehicle: EtOH/TritonX100/saline	F1 M/F Pups: PND 28–140, Adolescent, adult	↑Habituation of locomotor activity, Novelty suppressed feeding: ↓latency to begin eating; ↓Visual operant signal. Significance: Impaired operant attention into adulthood	2*	118
SD Juvenile M/F: PND 10–16. Dose: Gavage 10 mg/kg/day. Vehicle: corn oil	F1 M/F Pups: PND 29 & 38, Adolescent	↓Bodyweight gain; High Illumination: ↑entries & time in open arm; Low Illumination: ↑stretch attend posture; ↑head dips; ↓exploration, ↑frequency of nape attacks; ↑time & frequency play fighting. Significance: Altered social behavior in adolescence.	10*	137

(continued)

Table 2. *(continued)*

Animal strain/Sex/Duration/Dose/Vehicle	Day tested	Effects	LOEL (mg/kg/day)	Reference
		$^{\Delta 9}$THC in animal studies		
C57BL/6J Mice M Pup: PND 5–16 & 5–35. Dose: s.c. 1.0, 5.0 mg/kg/day. Vehicle not stated	F1 M Pup: PND 16 or PND 35 Preweaning, adolescent	Hippocampal cell rearranged CB1R; changes key molecular constituents of mitochondrial respiratory chain; Thinning of pyramidal cell layer; Neurochemical deficits Significance: Developmental deficits from neuronal disorganization, misrouted differentiation & associated pathologies.	1.0	119
Adolescent Treatment				
Long-Evans M PND 28 each 3rd day to PND 50. Dose: i.p. 1.5 mg/kg/day. Vehicle: saline/H_2O/Tween80	M: PND 50 or PND 63, Adolescent, adult	Adolescent: Disrupted development of dendritic arbors PFC (pyramidal neurons); Adult: prolonged atrophy in distal apical arbors of PFC neurons; Prematurely pruned dendritic spines attenuated neuroplasticity. Significance: Disrupted PFC neural networks lead to decreased cognitive & emotional dysregulation & affected decision making similar to pathology in human schizophrenia	1.5*	29
Long-Evans F PND 35–75. Dose: i.p. 5.6 mg/kg/day. Vehicle: saline	F: PND 75–160 & 159 to 200 Adult	Adult: ↑CB1R density; Persistent impairment of working memory & task performance. Significance: Long term effects on operant learning	5.6	123
Long-Evans M/F "Puberty Onset" for 14 days. Dose: i.p. 5 mg/kg/day. Vehicle: EtOH/Cremophor/saline	M/F: Day 14 treatment	M/F combined: ↓Total attacks, total pins, percent defense & complete rotation.	5.0 (only dose)	138
Wistar M i.p. 1.0 mg/kg/day PND 28–30 →5.0 mg/kg/day alternate days PND 34–52 or PND 60–62 →5.0 mg/kg/day alternate days PND 66-84 or Acute: 5 mg/kg/day: PND 52. Vehicle: Tween80/saline	M: PND 52, 55, 67, 70, 71, 72, 84, 87, 99, 102, 103, 104, Adolescent, adult	Adolescent: ↑Latency to emerge; ↓time in open areas; ↓rearings; ↓novel object preference; ↑memory deficits; alterations in hippocampal structure/function remaining to adulthood. Significance: Hippocampal alterations lead to persistent memory deficits that developed in adolescence	1.0	120
SD M/F PND 35–37; 5; 38–41; 10; 42–45. Dose: i.p. 2.5 mg/kg/day, twice/day. Vehicle: EtOH/cremophor/saline	M/F: PND 75: Adult	Adult: ↓ Bodyweight & food intake; ↓CB1R binding & stimulation (NAc, amygdala, VTA, hippocampus); ↓sucrose preference (anhedonia); ↓CREB activation in prefrontal cortex, NAc, hippocampus; ↑ dynorphin (indicates depression). Significance: Disruption of neural circuitry related to emotion and depression during adolescence	5.0	34
SD M PND 35–37; 5; 38–41; 10; 42–45. Dose: i.p. 2.5 mg/kg/day, twice/day. Vehicle: EtOH/cremophor/saline	M: PND 75 Adult	Adult: ↓Radial maze learning; ↓dendritic length in hippocampal dentate gyrus; ↓spine density; ↓NMDA receptors & biomarkers indicating ↓neuroplasticity. Significance: Spatial memory & cognitive deficits	5.0	122
CD1 Mice M PND 28–48; 69–89. Dose: i.p. 3.0 mg/kg/day. Vehicle EtOH/chermophor/saline	PND 49–53 & PND 90–94 Adolescent. PND 90–94 & PND 131–135 Adult	Adolescent: Impaired object recognition/working memory (novel object recognition & discrimination); repetitive/compulsive behaviors (↑percent shredded in nestlet; ↑marble burying); ↓delayed anxiety to move out of the dark; Adult: ↓novel object recognition performance; elevated plus maze ↓ anxiety to venture out. Significance: Behaviors common to those seen in animal schizophrenia models & humans	3.0*	121
Adult treatment				

(continued)

Table 2. *(continued)*

Animal strain/Sex/Duration/Dose/Vehicle	Day tested	Effects	LOEL (mg/kg/day)	Reference
		$^{\Delta 9}$THC in animal studies		
Long-Evans M. Dose: Acute i.p. 1.0, 1.5, 2.0 mg/kg. Vehicle: detergent/EtOH/saline	~15 min time increments postdose	↓Attention; ↓hippocampal functional cell types. Significance: Information not likely to be encoded correctly & unlikely to be accurately retrieved or recalled	0.5	127
Long-Evans M. Dose: Acute i.p. 0.01, 1.0 mg/kg. Vehicle: Tween80/saline	30 min postdose	↑Trials to achieving reversal task between stimulus & reward; affects c-fos expression associated with negative behavioral effects (orbital limbic & striatal regions in brain). Significance: Effects in orbitofrontal cortex & striatum (potential inelasticity) leading to an inability to perform reversal discriminations	1.0	124
Wistar M: 5 days. Dose: i.p. 2.0, 4.0 mg/kg/day. Vehicle: Tween 80/saline	30 min postdose	↓Short-term memory & discrimination affected by eCB increase at the CB1R. Significance: Disrupted CB1Rs is detrimental to memory & cognition	2.0	139
Wistar M: i.p. 7 days per dose. Dose: i.p. 1.0, 3.0, 10 mg/kg/day. Vehicle: EtOH/Tween 80/saline	20 min postdose	↓ Body weight; Anxiety measures: ↓time spent in emergence test; ↑ hide time; ↓ open field time; ↓percent open arm time; ↓active time; ↓total social interaction time & distance traveled; Place conditioning: ↓preference for the conditioned side; ↓CB1 R binding in hippocampus, substantia nigra, caudate putamen, cigulate gyrus. Significance: Affected anxiety, learning, memory & social interaction due to disruptions in CB1R binding in critical brain regions	1.0, 3.0, 10	129
SD M 2 times/day for 14 days. Dose: i.p. 5.0 mg/kg twice per day. Vehicle: Tween 80/saline	Post terminal dose	↓Performance attention, executive functions, memory, cognition associated with ↓DA in PFC. Significance: Disruption of the cortical dopaminergic pathways lead to cognitive & attention dysfunction	20	140
SD M: Acute (1 treatment). Dose: i.p. 5.0 mg/kg. Vehicle: OH-β-cyclodextrin/saline	30 min postdose	↑Working memory impairments; ↑DA turnover (DOPAC/DA); ↑NE turnover PFC. Significance: Cognitive impairment	5.0*	141
C57BL/6JArc mice M: 1 or 21 days. Acute & Chronic Doses: i.p. 0.3, 1.0, 3.0, 10 mg/kg. Vehicle: EtOH/Tween 80/saline	Acute & chronic 60 min postdose	Acute & chronic: ↑analgesia & catalepsy; ↓ thermic response & locomotor activity; Anxiety: ↓ distance traveled light/dark; ↓ frequency of entries in elevated + maze; ↓ vertical activity, rearing & head dipping; ↓startle response; ↓passive avoidance/anogenital sniffing, social interaction; ↑latency passive avoidance; ↑prepulse inhibition. Significance: Effect on neurotoxicity (anxiety) occurs after both acute & chronic exposure	1.0	126
CD1 mice M. Dose: Acute i.p. 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12, 48 mg/kg/day. Vehicle: EtOH/CremophorEL/saline	30 min postdose	↑Percent time in the open arm in the elevated plus maze; ↓anxiety; ↑percent swim time; ↑closed arm entries. Significance: ↓Anxiety & depression behaviors	0.8	125

ACh, acetylcholine; 2-AG, 2-arachidonoylglycerol; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CB1, cannabinoid 1 receptor; CHaT, choline acetyltransferase; CREB, cyclic adenosine monophosphate response element-binding; DA, dopamine; DAGL, diacyglycerol lipase; DOPAC, L-3,4-dihydroxyphenylacetic acid; DRD, dopamine receptor; eCB, endocannabinoid; F, female; GABA, gamma-amino butyric acid; GD, gestation day; GAD67, glutamic acid decarboxylase 67; GLUT, glutamate; H3C, hemicholinium-3; i.p., intraperitoneal; i.v., intravenous; LOEL, lowest-observed-effect level; LTD, long-term depression; M, male; MAGL, monoacylglycerol lipase; NAc, nucleus accumbens; NE, norepinephrine; PENK, preproenkephalin; PFC, prefrontal cortex; PND, postnatal day; PPI, prepulse interval; s.c., subcutaneous; SD, Sprague-Dawley; VTA, ventral tegmental area; ↓, decrease; ↑, increase; →, leads to; *, only one dose was used in the study.

amniotic fluid volume throughout pregnancy and decreased placental perfusion (oxygen availability decreases) accompanied by increased placental microinfarctions. In addition, there were significant changes in the RNA signature sequences in the placental transcriptome. These data indicate that disruptions in vascular development and angiogenesis affect the offspring through decreased testes weights and relative heart weights. Adult male rhesus macaques were treated with $^{\Delta 9}$THC in a cookie at 0.5 mg/7 kg/day (1–70 days), 1.0 mg/7 kg/day (71–140 days), and 2.5 mg/7 kg/day (141–210 days). At 210 days, there were dose-related decreases in testicular and epididymal weights.[170] Follicle-stimulating hormone, luteinizing hormone, and prolactin were increased, and total testosterone and estradiol were decreased. These effects indicate potential disruption of the hypothalamus–pituitary–gonadotropin axis, impacting testicular function.[171] In another study, adult female rhesus macaques were treated with $^{\Delta 9}$THC in a cookie at 0.5 mg/7 kg/day (1–3 weeks), 1.0 mg/7 kg/day (4–6 weeks), 2.0 mg/7 kg/day (7–9 weeks), and 2.5 mg/7 kg/day (10–12 weeks). At 12 weeks, the animals showed increases in menstrual cycle length and increased follicle-stimulating hormone concentrations, another indication of hypothalamus–pituitary–gonadotropin axis disruption.[171] The disruptions in hormonal balance, menstrual cycle, and ovulatory function would likely affect fecundity.[172]

$^{\Delta 9}$THC-associated effects in humans

A review by Frau and Melis[173] provides evidence showing that *in utero*, transplacental $^{\Delta 9}$THC exposure deregulates the mesolimbic dopaminergic system in males, potentially predisposing them to schizophrenia. Prenatal exposure in humans can act to prime the sensorimotor gating development in the brain, primarily in the VTA region associated with the dopaminergic system. Subsequent environmental exposures such as $^{\Delta 9}$THC or other stressors can lower the threshold to initiation of psychotic-like effects.[134] In addition, $^{\Delta 9}$THC exposure to infants during breastfeeding can continue more than 6 weeks after the last maternal consumption, potentially affecting brain development.[9,38,142,174] Monfort, Ferreira, Leclair, and Lodygensky[22] have described the pharmacokinetics of cannabinoid exposures during pregnancy, in infants, and during breastfeeding. While consumption may be due to depression, anxiety, nausea, or pain, data indicate that there are significant irreversible risks to neuronal development in fetuses, neonates, and the developing young.[22] Data also support the increased risks of dysregulated glucose-insulin measurements as well as obesity in children after maternal use of cannabis during pregnancy.[175]

Although $^{\Delta 9}$THC (cannabis) is not federally legal in the United States, acute and repeated human exposure to $^{\Delta 9}$THC is regulated by the European Food Safety Authority.[176] Human data were used by this agency to establish a lowest-observed-adverse-effect level (LOAEL) for an administered $^{\Delta 9}$THC exposure of 2.5 mg/kg/day (corresponding to an internal dose of 0.036 mg/kg/day). Applying an uncertainty factor of 3 to extrapolate from a LOAEL to a no-observed-adverse-effect level (NOAEL) and 10 for intraspecies differences produced 1 µg/kg/day (acute reference dose: ARfD = [0.036 mg/kg/day ÷ 30] = 1 µg/kg/day). However, it is evident from gestational treatment in Table 2 that offspring experienced neurodevelopmental effects related to motivation/reward, stress response, and increased sensitivity to opiate reward in adulthood at 0.15 mg/kg/day.[112,114] Establishing an ARfD would require the same uncertainty factors in addition to an interspecies default of 10 ([LOAEL 0.15 mg/kg/day ÷ 3 = NOAEL 0.05 mg/kg/day] ÷ [10 interspecies × 10 intraspecies]) = 0.5 µg/kg/day.[177–179] Gestational exposure to $^{\Delta 9}$THC may need a different ARfD than that of adults, since effects occur at very low doses. This is especially critical to re-evaluate because the low-dose animal studies used only one dose, and there were no doses below 0.15 mg/kg/day in which effects might also be seen in developing fetuses.

CBD-associated mechanisms

While it can make up as much as 40% of cannabis extract,[180] CBD has been purified in products for use by people and even their pets. CBD is one of the most actively studied therapies for a broad spectrum of neurological, inflammatory, and mental diseases because of its efficacy, low toxicity, and availability (e.g., over the counter and online order). The exact mechanism for the therapeutic effects is still under investigation,[42,181] but the proposed MOA for CBD indicates several targets associated with neuroprotection (Fig. 2[182] and Table 3).[86,183,184] Like $^{\Delta 9}$THC, CBD has effects on many interacting targets, and there is evidence for direct and indirect CBD actions on inflammatory and neurological parameters.[180]

Glial cells

CNS connective tissue (e.g., macroglia: astrocytes and microglia) consists of nonneuronal cells that link neuronal cells to the blood supply (blood–brain barrier), regulate blood flow to the brain, and regulate neurotransmission (macroglia) or serve as macrophages to mount immune responses in the brain (microglia).[185] When neuronal injury occurs, astrocytes can signal microglia to initiate an immune response; however, when the immune response becomes unbalanced, neuronal injury will occur.[181] CBD can decrease the microglial immune response to injured dopaminergic neurons in diseases like Parkinson's disease, and it increases the recruitment of astrocytes to promote neuronal regeneration through brain-derived neurotrophic factor (BDNF) (Table 3).

Adenosine receptor 2A ($A_{2A}R$)

Adenosine acts at a G protein-coupled receptor ($A_{2A}R$) on neuronal membranes to suppress immune responses due to inflammation or cell stress. CBD serves as an agonist to decrease adenosine reuptake, thereby increasing adenosine signaling and decreasing neuroinflammation.[186,187] CBD exposure decreases proinflammatory cytokine interleukin (IL)1β, microglial activity, tumor necrosis factor-alpha (TNFα), cyclooxygenase-2 (COX2), and inducible nitric oxide synthase (iNOS) activity in the brain (Table 3). These pathways have been shown to improve the effects of multiple sclerosis, hypoxic-ischemic brain damage, Alzheimer's disease, and hepatic encephalopathy.[183]

5-HT receptors

The dorsal raphe nucleus (DRN) is the primary serotonergic center (5-HT) in the brain where GCPR 5-HT_{1A} receptors are expressed. Receptor stimulation inhibits voltage-gated Ca^{2+} channels, activates K^+ channels, and inhibits neurotransmission in the DRN.[44,188] CBD has an anxiolytic effect by acting through 5-HT_{1A} receptor in male Wistar rats, previously stressed by foot shocks or restraint, but it can also induce anxiogenic behaviors in rats experiencing contextual fear conditioning,[182–190] perhaps by serving as an agonist at the 5-HT_{1A} receptor.[188] Acting on the serotonergic system, CBD is associated with improved locomotor activity (after striatal damage), cognition, cerebral ischemia, seizure disorders, and hepatic encephalopathy (Table 3).[183] Through the 5-HT1A receptor, CBD is associated with antiepileptic, anticataleptic, neu-

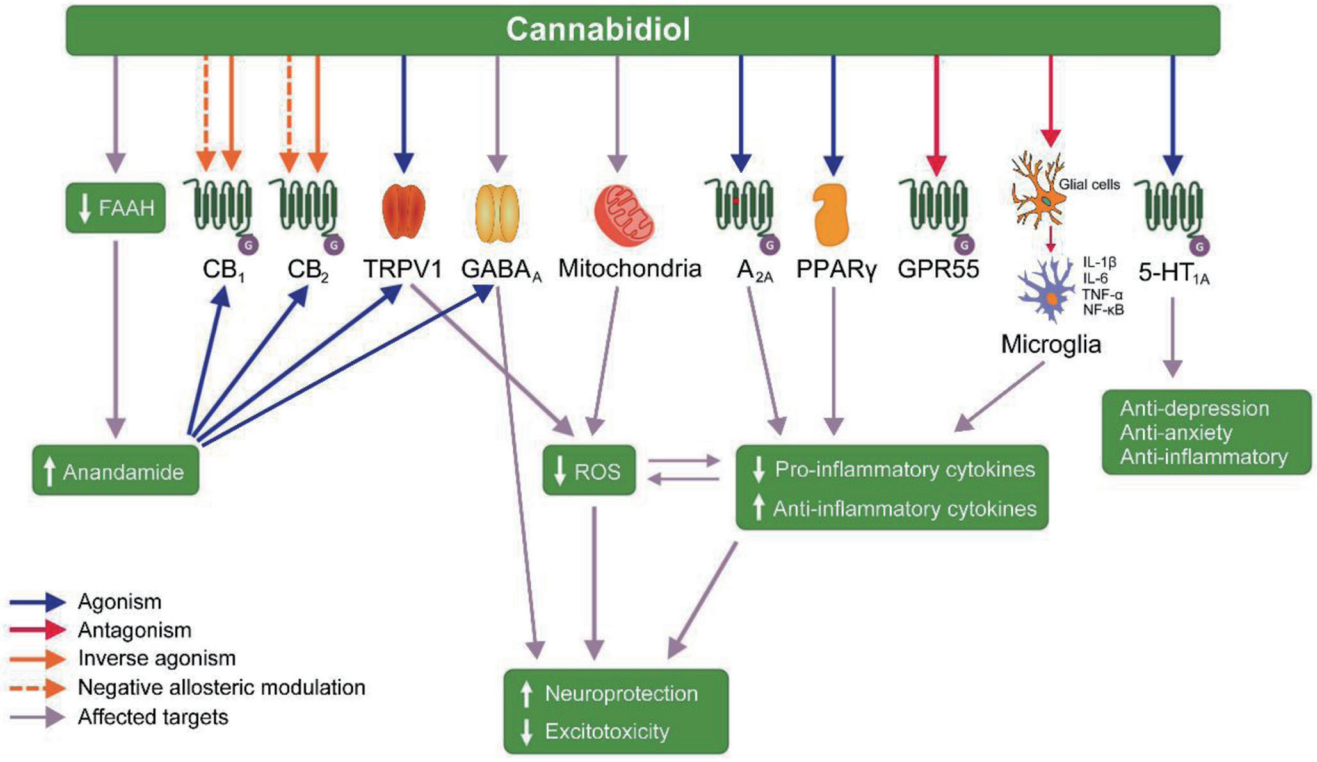

Fig. 2. The cannabidiol (CBD) mechanism of action includes: (1) agonistic activity toward the transient receptor potential vanilloid type 1 (TRPV1), the peroxisome proliferator activated receptor γ (PPARγ), and the serotonin$_{1A}$ (5-HT$_{1A}$) receptor; (2) antagonist activity at the G-protein coupled receptor GPR55; (3) antagonist to CB1 and CB2Rs in addition to acting as a reverse agonist and negative allosteric modulator; (4) antagonist of FAAH leading to increased anandamide (AEA), which goes on to activate the CB1, CB2, and TRPV1 receptors.; (5) direct action on the GABA$_A$ receptor (also influenced by AEA), leading to neuroprotection; (6) increased mitochondrial activity leading to antioxidant and anti-inflammatory action. Overall CBD has anti-depressant, anti-anxiety, and anti-inflammatory effects. Figure adapted with permission: Copyright © 2018.[182]

roprotective, antiemetic, anxiolytic, antidepressant, antipsychotic, and analgesic effects.[86,191–195] Others have also indicated that CBD acts via a negative allosteric mechanism in DRN somatodentritic 5-HT$_{1A}$ receptors that does not require CB1, 5-HT$_{2A}$, or GABA$_A$ receptors.[86,186]

CB1Rs and CB2Rs

CBD at the CB1Rs regulate excitotoxicity by inhibiting glutamate release to the NMDA receptors and normalizing glutamatergic activity. CBD acts to increase the blood supply to areas after ischemic incidents by decreasing endothelial-derived endothelin-1 or nitric oxide to increase vasoconstriction.[197] Neurodegeneration occurs with activation of microglial cells (immune cells in the brain); however, CB1R activation by CBD leads to a decrease of TNFα and IL12 and an increase of IL10. Activation of CB2 then decreases the proliferation and migration of microglial cells while decreasing TNFα by inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB; Table 3).[198,199] The anti-inflammatory action of CBD has been shown to improve neuronal damage from ischemic stroke, Tardive dyskinesia, and Parkinson's disease.

FAAH

CBD can act indirectly at the CB1R through inhibition of FAAH and the AEA transporter, leading to increased AEA and activation of CB1R.[200,201] Increased CB1R agonism leads to decreased eCB degradation and transport (Table 3).

TRPV1

TRPV consists of a vanilloid channel on the plasma membrane, considered by some to be a CB3R,[51] that induces neuropeptide release associated with pain perception, neuroinflammation, and body temperature regulation.[200] CBD at TRPV-1 channels leads to increased Ca^{2+} levels, resulting in desensitization and subsequent decreased pain. TRPV1 binding decreases microglial activation and migration as well as oxidative stress (Table 3). In addition, CBD can increase AEA levels by inhibition of FAAH.[202] However, AEA and CBD are both TRPV1 channel agonists. TRPV1 channel activation by CBD presynaptically increases glutamate release in the brain, which may serve to counteract/antagonize the inhibitory action of CB1R binding by CBD on colocalized glutamatergic neurons. TRPV1 activation by CBD agonism can increase the PI3K/Akt pathway signaling to decrease the incidence of hallmarks of Alzheimer's disease.

G-coupled protein receptor 55 (GPR55)

GPR55 binding protects against excitotoxicity potentially through GABA$_A$ receptor. CBD, as an antagonist, decreases GPR55 activation in the CNS to regulate such processes as neuropathic pain and antiepileptic activity.[203] CBD has a high affinity for GPR55, resulting in a decreased glutamate release in the hippocampus, thus causing anti-convulsive effects, also seen in human subjects.[180] Moreover, the use of CBD has been shown to result in improved Parkinson's disease and Dravet syndrome (DS) symptoms (Table 3).[183,204]

Table 3. ***In-vivo*** **and** ***in-vitro*** **examples of neuroprotective effects of CBD in different neurological diseases[183]**

Model	CBD dose	Treatment	Biological/pharmacological effect	Neurological disease
Neuroprotection through activation of A_{2A}Rs				
SJL/J mice: F	5.0 mg/kg, i.p.	Days 1–7 post infection	Microglia activation attenuated, downregulating the expression of VCAM1, CCL2 and CCL5 & proinflammatory cytokine IL1β. CBD improved motor deficits in the chronic phase of the disease	Multiple sclerosis
Newborn C57BL6 mice: M/F	0.1–1,000 μM	15 min pre-incubation	↓Acute brain damage & apoptosis; ↓glutamate concentration, IL6 & expression of TNFα, COX2, and iNOS	Hypoxic-ischemic brain damage
Primary rat microglial & N13 microglial cells & C57Bl/6 mice: M/F	20 mg/kg, i.v.	1/day for 7 days; 3 days/ week for 2 weeks	Inhibited ATP-induced intracellular Ca^{2+} increase in cultured N13 & primary microglial cells and A_{2A} receptors may be involved in this mechanism. *In vivo:* ↓gene expression of proinflammatory cytokine IL6 & prevented cognitive impairment induced by βA	Alzheimer's disease
Sabra mice: F	5.0 mg/kg, i.p.	28 days	↓Hippocampal TNFα-R 1 gene expression but ↑expression of the BDNF gene. Indirect activation of A_{2A}R, ↑cognitive & motor function in rats with hepatic encephalopathy.	Hepatic encephalopathy
Neuroprotection through the activation of the 5-HT_{1A}				
MCA occlusion mice: M	3.0 or 10 mg/kg, i.p.	Before & 3 h after damage	CBD significantly ↓infarct volume induced by MCA occlusion through 5-HT_{1A} receptor	Cerebral ischemia
Swiss mice: M	5.0, 15, 30, or 60 mg/kg, i.p.	30 min before receiving drugs to induce catalepsy	CBD pretreatment ↓catalepsy in a dose-dependent manner, through the 5-HT_{1A}R	Striatal disorders
Swiss mice: M	15–60 mg/kg or 60 nmol, i.p.	30 min before or 2.5 h after receiving the drugs to induce catalepsy	CBD pretreatment ↓catalepsy in a dose-dependent manner, through the 5-HT_{1A}R	Striatal disorders
Wistar Kyoto rats: M	100 mg/kg	60 min before seizure induction	CBD significantly mitigated PTZ-induced seizure	Seizure disorders
Adult Wistar rats: M	0.1–1.0 mg/kg & 5.0 mg/kg, i.p.	Acute treatment + cumulative injections every 5 min & repeated at 5 mg/kg/day for 7 days	CBD protected nerve injury-induced deficits in dorsal raphe nucleus 5-HT neuronal activity & exerted antiallodynic effects by TRPV1 activation & anxiolytic properties through 5-HT_{1A} receptor activation	Allodynia & anxiety
Sabra mice: F	5.0 mg/kg, i.p.	28 days	CBD, by 5-HT_{1A}R activation, ↑cognition & motor function, impaired by bile-duct ligation. CBD ↓neuroinflammation, ↑BDNF gene expression & ↓TNFαR 1 gene expression in hepatic encephalopathy model	Hepatic Encephalopathy
Sabra mice: F	5.0 mg/kg, i.p.	Single acute dose	CBD ameliorated cognitive deficits & locomotor activity; restored brain 5-HT levels & improved liver function	Hepatic Encephalopathy
C57BL/6J mice: M	30 mg/kg/day, i.p.	7 days	↑Time spent interacting; ↓psychotic-like behaviors acting through 5-HT 1A receptors	Schizophrenia
Neuroprotection by antagonistic activation of GPR55				
Scn1a mutant mice (DS model): M/F	10, 20, 100, or 200 mg/kg/day	Twice/day for 7 days	Acute CBD ↓thermally induced seizures & ↓spontaneous seizure rate. Low doses ameliorated autism-type social interaction deficits in genetically induced DS model, ↑GABA inhibitory transmission impaired in DS mediated by GPR55	DS

(continued)

Table 3. *(continued)*

Model	CBD dose	Treatment	Biological/pharmacological effect	Neurological disease
Adult C57BL/6 mice: M	5.0 mg/kg	5 days/week, 5 weeks	↓Density of microglial cells in the cell body. In the haloperidol-induced catalepsy model, through GPR55-activation.	Parkinson's disease
C67BL/6 mice M/F	5.0–10 & 50 mg/kg	Increasing doses from 5.0 to 10 mg/kg 3 times/ week, or daily, at 50 mg/kg, for 23 days	EAE disease ameliorated (all doses), ↓encephalitogenic cell vitality, ↓levels of IL6, production of ROS, ↓apoptosis & GPR55R in CNS	EAE disease
Neuroprotection through activation of the TRPV receptors				
Wistar rat: M	10 mg/kg, i.p.	2 h after the induction of model	CBD inhibited carrageenan-induced hyperalgesia by desensitization of TRPV1R	Hyperalgesia
hPBMECs & hCMEC/ D3 Cells[a]	0.1, 0.3, 1.0, 3.0, 10, 15 µM	7 or 24 h of incubation	Dose-related ↑in intracellular Ca^{2+} through activation of TRPV2 enhanced cell proliferation, cell migration & tubulogenesis in human brain endothelial cells.	—
U87MG Human glioblastoma cell line	10 µM	Cells treated with different CBD doses 1 day or co-treated with CBD 10 µM & chemo drugs 6 h	TRPV2 activation & ↑Ca^{2+} improved chemotherapy drug action by enhancing absorption & ameliorating cytotoxic activity in human glioma cells	—
Human gingival mesenchymal stem cells	5 µM	24-h incubation	TRPV1 desensitization promoted the PI3K/Akt pathway[b] signaling, which can reduce Alzheimer hallmarks	Alzheimer's disease
Neuroprotection through the activation of the PPARγ				
SH-SY5Y^{APP+} cells	10^{-9}–10^{-6} M	24 h	↓Expression of amyloid precursor protein & its ubiquitination, leading to ↓Aβ & neuronal apoptosis. Effects mediated by PPARγ activation	Alzheimer's disease
Primary rat astrocytes & SD rat: M	10^{-9}–10^{-7} M: *in vitro;* 10 mg/ kg: *in vivo*, i.p.	15 days	*In vitro:* Dose response ↓ in Aβ mediated through inhibition of NF-κB; Aβ-induced neuronal damage led to ↓gliosis & glial fibrillary acidic protein. Effects exerted through PPARγ activation	Alzheimer's disease
Hippocampal slices from C57Bl/6 mice	10 µM	30 min before addition of Aβ	Improved synaptic transmission & long-term potentiation in the hippocampus slice of C57BL/6 mice, protecting it from cognitive deficits induced by Aβ 1–42. CBD effects exerted through interaction with PPARγ	Alzheimer's disease
Newborn C57/ BL6 & Swiss mice primary microglial cultures: M/F	60 mg/kg: *in vivo,* i.p.; 10 µM: *in vitro*	2 injections/day 30 min prior to haloperidol: 21 days	Dyskinesia prevented after induction haloperidol. ↓Oxidative stress in corpus striatum, ↓activation of microglial, inflammatory cytokine (e.g., IL1β and TNFα), ↑anti-inflammatory cytokine IL10. CBD affects PPARγ actions on lipopolysaccharide-stimulated microglial cells	Tardive dyskinesia
Adult C57/BL6 mice: M	15, 30, or 60 mg/kg, i.p.	15 min before L-DOPA administration for 3 days	CBD did not prevent L-DOPA-induced dyskinesia. Cotreatment of CBD + capsazepine, acting through CB1R & PPARγ, ameliorated dyskinesia.	Parkinson's disease

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Table 3. *(continued)*

Model	CBD dose	Treatment	Biological/pharmacological effect	Neurological disease
Human brain microvascular endothelial cell/human astrocyte co-cultures	100 nM, 1.0 & 10 µM	Before or directly after induction of ischemic damage	10 µM prevented enhanced BBB permeability after ischemic damage induced by oxygen-glucose deprivation, through by activating PPARɣ & $5HT_{1A}R$.	Ischemic stroke
Neuroprotection through positive allosteric modulation of $GABA_A$ receptors				
Surgical human DS & TSC cortical tissue in Xenopus oocytes	5.0 µM	Pre-incubation of cells 10 s before co-application of GABA & CBD	Positive modulation of $GABA_AR$, ↑amplitude of GABA-evoked current in brain tissues of patients with DS &TSC	DS & TSC
Scn1a+/- mice (M/F) & Xenopus oocytes expressing $GABA_A$ receptors	*in vivo* 12 or 100 mg/kg, i.p.; *in vitro* 10 µM	*In vivo*: CBD administered i.p. 45 min before CLB. *In vitro*: CBD (10 µM) co-applied with GABA, for 60 s	↑CLB concentration & active metabolite N-CLB in plasma & brain. Co-administration ↑anticonvulsant effect by enhancing the activity of the $GABA_A$ receptor	DS

[a]hCMEC/D3 cells: Human hematopoietic stem-cell-derived cells (HBLECs) and human primary brain microvascular endothelial cells (hPBMECs) used in BBB models. [b]PI3K/Akt pathway: Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt kinase phosphorylation involved in the cell cycle is decreased in the Alzheimer's brain (associated with amyloid-β and tau pathologies).[184] βA, β-amyloid; A2AR, adenosine 2A receptors; ATP, adenosine triphosphate; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; CBD, cannabidiol; CB2R, cannabinoid receptors type 2; CCL-2, chemokine ligand 2; CCL-5, chemokine ligand 5; CLB, clobazam; N-CLB, N-desmethylclobazam; CNS, central nervous system; COX2, cyclooxygenase-2; DS, Dravet syndrome; F, Female; GABA, ɣ-aminobutyric acid; GPR55, G-coupled protein receptor 55; hPBMECs, human primary brain microvascular endothelial cells; IL1β, interleukin 1β; IL6, interleukin-6; IL17, interleukin-17; iNOS, inducible nitric oxide synthase; L-DOPA, l-3,4-dihydroxyphenylalanine; M, male; MCA, middle cerebral artery; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PPARɣ, peroxisome proliferator activated receptor-ɣ; PTZ, pentylenetetrazole; ROS, reactive oxygen species; Scn1a+/-, heterozygous loss of function SCN1A; SD, Sprague-Dawley rat; SH-SY5Y^{APP+}, SH-SY5Y cells transfected with the amyloid precursor protein; TNFα, tumor necrosis factor α; TSC, tuberous sclerosis complex; TRV1 or 2, transient receptor potential vanilloid type 1 or 2; VCAM1, vascular cell adhesion molecule-1. Table adapted with permissions: Creative Commons — Attribution 4.0 International — CC BY 4.0.[86,183]

Peroxisome proliferator-activated receptor gamma (PPARɣ) receptors

CBD is an agonist of PPARɣ, a nuclear receptor and ligand-inducible transcription factor that produces anti-inflammatory and antioxidative effects.[199] PPARɣ modulates inflammation by inducing ubiquitin-proteasomal degradation of p65, resulting in inhibition of proinflammatory gene expression of cyclooxygenase (COX2) and proinflammatory mediators (e.g., TNFα, IL1β, and IL6) in addition to inhibition of NFκB-mediated inflammatory signaling. CBD agonist activity with PPARɣ also contributes to the inhibition of TNFα, IL1β, and IL6 transcription to prevent NFκB signaling, and it also produces antioxidant properties.[198,199] It increases eCBs by antagonist activity at CB2Rs, and the eCBs then act as PPARɣ agonists to promote anti-inflammatory and antioxidant actions. Furthermore, Alzheimer's disease has been demonstrated to be improved via the PPARɣ-mediated protective effects of CBD (Table 3).

$GABA_A$ receptors

As the main inhibitory neurotransmitter in the CNS, GABA disruption is associated with neurological diseases, including cognitive deficits, drug addiction, chronic stress and anxiety, epileptic disorders, and Huntington's disease.[180,205] CBD stimulates GABAergic neurotransmission, meaning that the inhibitory neurotransmission and frequency are increased.[206] Seizure frequency, duration, and severity were reduced in addition to increased social behaviors in a mouse model of DS and other diseases after CBD treatment. In addition, overexcitation in the dentate gyrus of the hippocampus was decreased through CBD effects on $GABA_A$ receptors.[206] Therefore, with CBD bound to the $GABA_A$ receptor, anticonvulsant and anxiolytic actions are seen in the CNS. Moreover, since CBD does not bind competitively with the benzodiazepine receptor, it is potentially useful in patients resistant to benzodiazepines, which is the standard antiseizure treatment (Table 3).[207]

CBD-associated neuroprotection in animal studies

CBD has shown neuroprotective effects in animal models with several neural-associated disease states (Table 3).[86,181,183,208] The areas studied have focused mainly on neuroprotection and treatment of brain-related diseases (e.g., multiple sclerosis, Alzheimer's disease, and schizophrenia), rather than effects on other areas of the body (e.g., local pain). CBD at doses from 5.0 mg/kg/day in rodents has many beneficial effects (Table 3). Note that doses administered *in vivo* were by i.p.; therefore, CBD is more slowly absorbed and subject to local metabolic processes prior to entering the blood stream, as would occur with oral exposure.[107,111] Table 3 indicates pathways specifically shown to be associated with CBD exposure.

CBD neuroprotection in human studies

The neuroprotective effects of CBD observed in animal studies are supported by observations in human subjects. CBD is well tolerated in children and adults and has a broad spectrum of therapeutic benefits to help with significant neurological disease states,[209] including neurological damage and disorders, brain tumors, Parkinson's disease, Huntington's disease, Alzheimer's disease, multiple sclerosis, neuropathic pain, and childhood seizures (e.g., Lennox-Gastaut syndrome and DS).[180,210] Additionally, synthetic forms of CBD have been used to treat drug-resistant epilepsies in children

Table 4. Neuroprotection for Parkinson's disease initiated with cannabidiol treatment

CBD target	Biological effect
CBD neuroprotection in Parkinson's disease (review)[42]	
CB1 activation	↓Microglial activation and microglial NADPH oxidase expression; ↓Production of proinflammatory agents (IL1β, TNFα, iNOs, COX2); ↓Dopaminergic neuronal damage; ↓Excitotoxicity (↓glutamate release); ↓ROS and lipid peroxidation
CB1 antagonism	↑Astrocyte activation in substantia nigra pars compacta
CB2 activation	↓Microglia number and production of proinflammatory agents (IL1β, TNFα, iNOs, nitric oxide); ↓Dopamine depletion; ↓Myeloperoxidase-positive astrocytes; ↑Antioxidant enzyme activity and antioxidant agents
MAGL inhibition	↓Microglia and astrocyte number; ↑CB2 activation; ↑GDNF
FAAH inhibition	↑Motor activity; prevents excitotoxicity by inhibiting glutamate release due to neuroinflammation; ↓Protein carbonylation; ↓ROS and lipid peroxidation
PPARɣ activation	↓ROS
CBD neuroprotection in Huntington's disease (review)[42]	
CB1 activation	↓Excitotoxicity (↓glutamate release)
CB2 activation	↓Reactive microglial cell number; ↓Production of proinflammatory agents (TNFα); ↓ROS and nitric oxide; ↑Production of neurotrophins & anti-inflammatory mediators (IL10, IL1 antagonist)
Phytocannabinoid structure	↓ROS (phenolic structure acts as an ROS scavenger)
PPARɣ activation	Interference with the NFκB signaling pathway; Induction of antioxidant enzymes
CBD neuroprotection in Alzheimer's disease (review)[42]	
PPARɣ activation	↓Apoptosis during neurodegeneration; ↓Astrocyte activation; ↓Expression of proinflammatory cytokine IL1β and iNOS (↓neuroinflammation); ↓Amyloid plaque and inflammation
CB1 activation	↓Amyloid β-induced memory impairment
CB2 activation	↓Proinflammatory mediators from microglial cells and astrocytes; ↓Neuroinflammation

CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; COX2, cyclooxygenase 2; FAAH, fatty acid amide hydrolase; GDNF, glial cell-derived neurotrophic factor; IL, interleukin; iNOS, inducible nitric oxide synthase; MAGL, monoacylglycerol lipase; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; TGF, transforming growth factor; TNF, tumor necrosis factor.

(age ≥2 and older) (Lennox-Gastaut syndrome or DS).[210] Epidiolex/Epidyolex (>99% CBD) is approved by the United States Food and Drug Administration and the European Medicines Agency to treat these diseases.[211] The benefits of CBD also have been shown in human subjects to treat anxiety, depression, post-traumatic stress disorder, and obsessive-compulsive disorders;[212,213] furthermore, it has demonstrated antipsychotic properties in those with schizophrenia.[214] A few examples of CBD affecting neurological diseases are listed in Table 4 (review).[42]

Parkinson's disease

The hallmark of Parkinson's disease is the accumulation of α-synuclein and the degeneration of dopaminergic neurons in the SNa in addition to motor alterations (bradykinesia, resting tremors, rigidity, and postural instability), depression, and dementia (review).[42] Improvement in the disease by CBD occurs via numerous pathways acting through the eCBS (e.g., CB1Rs, CB2Rs, FAAH, and MAGL) to modulate excitotoxicity, dopaminergic neuronal degeneration through inflammation, and microglial inhibition (Table 4).[43,202,215–217] Importantly, CBD has been used to improve the effects of Parkinson's disease in human subjects (review).[218]

Huntington's disease

Huntington's disease is an autosomal-dominant neurodegenerative disease that is progressive, leading to degeneration of striatal GABA and dopaminergic neuronal destruction in the globus pallidus.[43] CB1R activation by CBD in the striatum can inhibit glutamatergic transmission to protect damaged neurons and serve as an antioxidant (Table 4).[43,217,219,220]

Alzheimer's disease

CBD has been shown to decrease or block hyperphosphorylation of tau protein, acetylcholinesterase activity, oxidative stress, apoptosis, neuroinflammation, gliosis, and deposition and expression of beta-amyloid (βA).[210] The mechanism is associated with selective activation of PPARɣ, resulting in increased clearance of βA peptides through autophagy in the hippocampus, ubiquitination of amyloid precursor proteins, and decreased βA deposition (Table 4).[43,210]

CBD-associated toxicity

Since it is not considered to be intoxicating, compared to $^{\Delta 9}$THC, CBD has been widely used for medicinal purposes and is of great interest to medical communities.[17] While CBD use has increased in humans for a plethora of conditions, little is known about the potential for risks from consumption during pregnancy or in children using CBD to treat epilepsy.[17,221] The effects of CBD on brain development *in utero* are not well understood; however, C57BL6/J dams treated with 3.0 mg/kg s.c. GD 5-18 had pups with sex-specific behavioral effects (Table 5).[15–17,23,24,183,222,223,228], The male

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Table 5. Neurotoxic, behavioral, and reproductive effects from CBD treatment during development in animal studies

Animal strain sex/duration/dose/vehicle	Day tested	Effects	LOEL (mg/kg/day)	Reference
Gestational treatment				
In-vitro C57Bl/6J mouse whole embryos. 6 somite embryos for 24–30 h of culture. Dose: 0, 15, 30 µM CBD. Vehicle: EtOH	24–30 h	No effects on embryo growth. ↓cranial neural tube closure 15, 30 µM. Significance: Adverse effects on brain development *in vitro*	15 µM	23
C57Bl/6J mouse M/F: GD 5–18 S.C. Dose: 0, 3 mg/kg/day. Vehicle: Cremophor EL, EtOH, saline	PND 10 and 13	10d: Mean USV duration ↓ (M) and frequency ↑ (F); PND 10, 13, 16, 19, 22: ↓body weight (M); Syllabic repertoire of sound communication sex specific; ↓Homing behavior: Distance moved, velocity, movement distance moved from nest (F). Significance: Adverse neuronal development *in vivo*	3 mg/kg/day (only dose tested)	24
Postnatal treatment				
In Vitro Wistar primary neonatal (PND 2) rat cerebral cortices (astrocytes + neurons) 1–24 h. Dose: 0, 0.5, 1, 5 µM CBD. Vehicle: EtOH	24 h	Neuron: All doses tested: Viability ↓ LDH ↑ at ≥0.1 µM; Only 0.1 µM tested: Change in mitochondrial membrane potential, ↑ATP depletion & caspase 4/7 activation, ↑apoptosis & chromatin condensation; ↓dendrite length; Astrocytes: All doses tested: Viability ↓ LDH ↑ at ≥0.5 µM; Only 0.5 µM tested: dysregulated mitochondrial membrane potential, ↑ATP depletion & caspase 8, 9, 4/7 activation, ↑apoptosis & necrosis. Significance: Cytotoxic to neurons and astrocytes *in vitro*	Neurons: 0.1 µM; Astrocytes: 0.5 µM	17
In vitro 18-week-old human M: Sertoli cells mouse sertoli cell line. Dose: Human: 7, 8, 9, 10 µM. Mouse: 10, 12.5, 15, 17.5, 20 µM. Vehicle: DMSO	24 h	Human & Mouse: ↑Cytotoxicity & cell senescence; ↓DNA replication & DNA repair; disruptions in cell-cycle related genes; ↓Cell viability; inhibition of G1/S phase cell cycle transition; ↓mRNA for Wilms' tumor 1 biomarker. Significance: Adverse effects on human Sertoli cells *in vitro*	Human: 7.0 µM; Mouse: 10 µM	228
Adolescent treatment				
Swiss mice M: PND 21–55 (4 spermatogenic cycles), gavage. Dose: 0, 15 & 30 mg/kg/day. Vehicle: Sunflower oil	PND 90	↓Testosterone (30 mg/kg/day); ↓spermatogenesis (≥15 mg/kg/day); ↑sperm with head abnormalities & cytoplasmic droplets (≥15 mg/kg/day); affected seminiferous tubule morphology (≥15 mg/kg/day). Significance: Disrupted sperm development likely affected fertility	15 mg/kg/day	223
Swiss Mice M: PND 21–55 (4 spermatogenic cycles), gavage. Dose: 0, 15 & 30 mg/kg/day. Vehicle: Sunflower oil	PND 90	Germinal epithelium stages disrupted & seminiferous tubule dysmorphology during spermatogenesis (≥15 mg/kg/day); ↑malonaldehyde & ↓sperm motility, super oxide dismutase & catalase at 30 mg/kg/day; ↑abnormal acrosome reaction & sperm velocity (≥15 mg/kg/day). Significance: Potentially affected fertility & ↑oxidative stress	15 mg/kg/day	15
Adult CBD treatment				
Wistar rat: 1 treatment (M/F) or 4 days (F). Dose: 0, 0.3, 3, 30 mg/kg. Vehicle: Not stated	F: pro- and late diestrus 1 h/4 d; M: 1 h	Acute: Late diestrus ↑entries into & time spent in open arms EPM (0.3 mg/kg/day F; 3.0 mg/kg/day M); 4-day F: Late diestrus ↑entries & time spent into open arms EPM. Significance: Disrupted behavior, indicating neuronal damage in both sexes.	F: 0.3 mg/kg/day; M; 3.0 mg/kg/day	16

CNS, central nervous system; EPM, elevated plus maze; F, female; GABA, gamma-aminobutyric acid; GPR55, G-coupled protein receptor 55; hPBMECs, human primary brain microvascular endothelial cells; IL1β, interleukin 1β; IL6, interleukin-6; IL17, interleukin-17; iNOS, inducible nitric oxide synthase; L-DOPA, l-3,4-dihydroxyphenylalanine; LOEL, lowest-observed-effect level; M, male; MCA, middle cerebral artery; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PPARγ, peroxisome proliferator-activated receptor gamma; PTZ, pentylenetetrazole; ROS, reactive oxygen species; Scn1a+/-, heterozygous loss of function SCN1A; SD, Sprague-Dawley rat; SH-SY5Y^{APP+}, SH-SY5Y cells transfected with the amyloid precursor protein; TNFα, tumor necrosis factor alpha; TSC, tuberous sclerosis complex; TRV1 or 2, transient receptor potential vanilloid type 1 or 2; USV, ultrasound vocalizations; VCAM1, vascular cell adhesion molecule-1. Table adapted from the review by Silvestro, Schepici, Bramanti, and Mazzon,[183] with permissions: Creative Commons — Attribution 4.0 International — CC BY 4.0.

pups showed higher body weights, and there were effects on ultrasonic vocalizations (both sexes), homing behavior, and decreased motor and discriminatory abilities (females). These findings indicate that CBD has effects on psychopathology after *in-utero* exposure at 3.0 mg/kg/day and may not be as safe as previously considered when consumed during pregnancy.

In adults, aspects of CBD neurotoxicity are related to sex and strain in rodent studies.[208] For example, male and female Swiss and C57BL/6 mice were treated with a single dose of CBD at 0 (saline/Tween 80), 10, and 20 mg/kg/day, and Flanders-sensitive line rats and Flanders- resistant line rats were treated with CBD at 0, 10, 30, and 60 mg/kg/day i.p. The mice were tested in the elevated plus maze, which measures anxiety behavior, and in the tail suspension test, which measures immobility and antidepressant behavior) 30 min after treatment. There were no effects from treatment with either strain of females in the tests, but male Swiss mice showed increased immobility in the tail suspension test at all doses (antidepressant). In the elevated plus maze test, the female Swiss mice showed decreased entries into the enclosed arm, indicating decreased exploratory behavior (antidepressant-like effect). Meanwhile, male and female C57BL/6 mice did not show effects in the elevated plus maze test. Rats were also tested 50 min after treatment in the forced swim and open field tests. The Flanders-sensitive line rats showed decreases at all doses in the forced swim test (measure of immobility), with no effects on distance traveled in the open field test and no effects in these tests with Flanders-resistant line rats. When the interval between treatment and testing was increased to 2 h, there was a slight increase in immobility in the Flanders-sensitive line rats at 30 mg/kg CBD. Therefore, it is significant to note that the exposure time, sex, strain, and species differences with CBD treatment were related to anxiety/depressive behaviors. The doses used in this study and those shown in Table 5 are within the range of those showing neuroprotection in Table 3, also administered i.p. *In-vitro* studies with mouse embryos also support the toxic effects of CBD during development.[16]

Animal studies have shown that doses of CBD that are neuroprotective (Table 3), can be toxic to the male reproductive tract.[14,15,223–225] CBD treatment at 15 mg/kg/day (gavage) for three sperm development cycles in mice can lead to disrupted sperm development, abnormal seminiferous epithelium, decreased testes weights, and other effects that would impact fertility.[14] Studies also have demonstrated reduced testosterone, inhibition of sperm maturation, and thinning, atrophied cells, pyknosis in seminiferous tubules, and other pathologies.[14] The presumptive MOA involves CBD inhibition of 17α-hydroxylase in Leydig cells, leading to decreased testosterone production. However, in humans, the effects on sperm and other reproductive parameters in males have been mainly attributed to the $^{\Delta 9}$THC content in cannabis, rather than CBD.[226,227] But based on animal studies, CBD in cannabis could contribute to the negative effects in males; hence, this area needs more research. *In-vitro* studies performed on human and mouse Sertoli cells obtained postnatally support the toxic effects of CBD observed in animal studies.[18] Dose exposure, route, species, sex, frequency of consumption, and susceptibility to the effects from exposure contribute to health outcomes.

Future directions

With increasing use of cannabis with higher concentrations of $^{\Delta 9}$THC, there are concomitant risks to safety in the general population from intoxication while driving or in the workplace. Methods have been developed to measure impairment from cannabis in a timely manner on site (e.g., in a car or workplace) through brain imaging to provide assessments of intoxication.[39] Functional near-infrared spectroscopy provides a measurable signature of neural impairment of the PFC, and the results are supported by blood and urine assessments to indicate whether participants were exposed but not impaired or exposed and impaired. Such measures acknowledge the growing need for detection and mitigating safety measures due to cognitive impairment from cannabis use.

Neurotoxicity of CBD is also in need of more study. For example, CBD injured neonatal rat cortical neurons and astrocytes *in vitro* at low therapeutic levels that could affect patients treated with CBD.[17,221] CBD is known to be neuroprotective in Parkinson's disease, where dopaminergic neurons of the substantia nigra pars compacta are shown to degenerate.[78,194,229] Conversely, in animal models, dopaminergic pathways are attenuated by CBD, resulting in decreased motor functions.[24] While data indicate that for some, the benefits of CBD may outweigh the risks, it is clearly necessary to continue researching optimal treatment levels related to disease improvement. Persons exposed to higher doses of CBD for severe illnesses, such as DS to control seizures (Epidiolex®, Epidyolex® in Europe), may need to weigh the risk versus benefit and exert caution for use in pregnant women and children.

Finally, one of the biggest challenges in characterizing the effects of cannabis during developmental life stages is knowing the exposure and individual health risk factors. In laboratory experiments, the exact dose, purity of cannabinoids, animal strain/sex/pregnancy status, duration of exposure, and other parameters are controlled; however, with human subjects, it is difficult to characterize exposure. Nevertheless, knowledge of the dose and product components being consumed as well as the life stage of exposure, route of exposure (i.e., inhalation, s.c., i.v., oral, or i.p.), body fat composition, age, health status, frequency of use, and other factors will determine the absorption, distribution, and metabolism of cannabinoids entering the blood stream.[107,111] Many of these parameters are not consistent among studies performed in animals (e.g., different animal species/strains, dosing regimens, vehicles), and data may be difficult to obtain in epidemiological studies with human subjects. Thus, there is a need for further study to protect fetuses, infants, and children from harmful exposures during development. There is also a need for further research related to risks for male reproductive toxicity.

Conclusions

This review focused on neurotoxicity and neuroprotection of the most thoroughly characterized phytocannabinoids in cannabis—$^{\Delta 9}$THC and CBD. Most cannabis exposure is not a pure form of either compound, but it contains a combination of those and over 100 others. Due to the increasing use of cannabis or CBD, not just recreationally, but for the treatment of diseases (e.g., depression, anxiety, inflammation, pain, and seizures) and a plethora of other conditions, it is critical for the industry to thoroughly characterize expected exposures. The extent of the risk versus beneficial effects of compounds in cannabis is dependent on many factors, but, as indicated by studies with $^{\Delta 9}$THC, there is a high risk for long-lasting neurodevelopmental effects from exposure to fetuses, infants, children, and adolescents, including severe mental dysfunction (e.g., depression, anxiety, and schizophrenia), decreased cognition, drug dependency tendencies, and decreased motor function. Adolescent use can present unique challenges because adolescence is a developmental stage of increased independence and potential for experimentation with cannabis. In addition, brain development as

well as major dynamic changes in the eCBS continue for the first 25, or more, years of life; hence, cannabis exposure during adolescence can still attenuate brain development. Adolescent exposure has been shown to lead to persistent adverse neurodevelopmental changes, increasing the risks for major depressive disorder, drug addiction, and severe psychotic disorders.

On the other hand, CBD is nonpsychotropic and has positive therapeutic applications to treat childhood epilepsy, multiple sclerosis, stroke, Alzheimer's disease, Parkinson's disease, and other severe disorders. The focus has been mainly on the health benefits; however, the reported developmental effects from exposure *in utero*, effects on male reproduction, and associations with human genotoxicity have not been well studied, and a significant data gap remains.

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Conflict of interest

I have no competing interests (financial/personal) to declare.

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MHS is the sole author of this work.

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