The Endocannabinoid System (ECS)

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Learning Objectives

- Review the physiological function of the endocannabinoid system (ECS) as a homeostatic regulator in the body
- Discuss strategies to support the endocannabinoid system
- Understand the bioactivities of phytocannabinoids and terpenes, as one of the approaches to support ECS functions
- Review safety of phytocannabinoids

History of the ECS

1963 and 1964
Isolation and structure identification of THC and CBD

1988
Discovery of CB1 receptors in rat brains

1992
Discovery of the first eCB, AEA

1993
Discovery of CB2 receptors

1995
Discovery of the second eCB, 2-AG

1996
Discovery of the first cannabinoid-metabolizing enzyme, FAAH

2000s
- Discovery of other eCB and eCB-like compounds
- Cell biology and neuroscience studies
- Clinical trials initiated

1960s | 1980s | 1990s | 2000s

Image adapted from: Mechoulam R et al. *Nat Rev Neurosci*. 2014;15(11):757-764.

2-AG, 2-arachidonoylglycerol; AEA, anandamide; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; CBD, cannabidiol; eCB, endogenous cannabinoid; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; THC, tetrahydrocannabinol.

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Role of the ECS

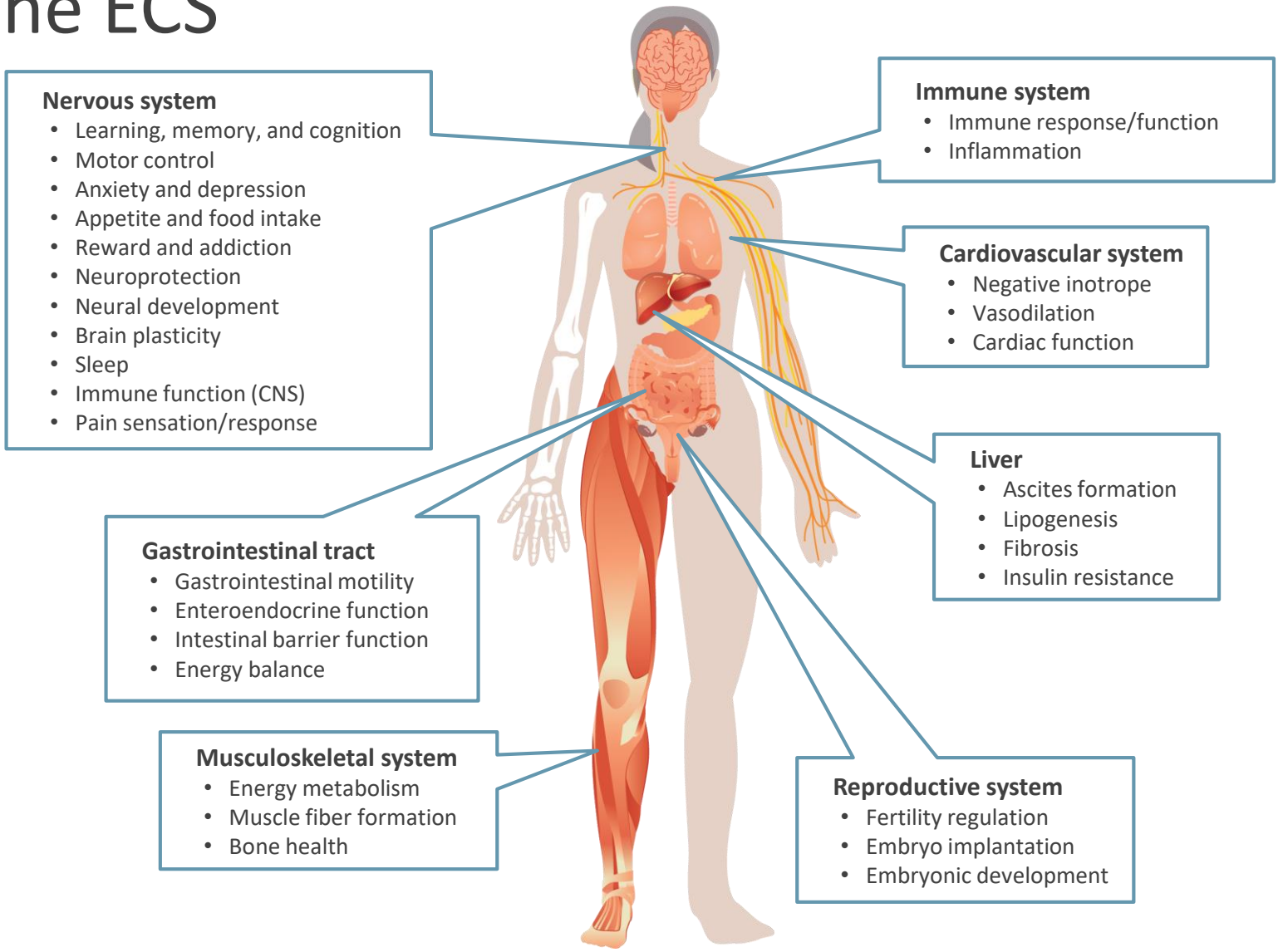

Image adapted from: Zou S et al. *Int J Mol Sci*. 2018;19(3):e833.

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The ECS Comprises Three Main Elements

1 Receptors

- CB1, CB2, $TRPV_1$, GPR55, PPAR

2 Endocannabinoids (eCBs)

- 2-AG, AEA, virodhamine, NADA

3 Enzymes

- Biosynthetic: NAPE-PLD (AEA);
  DAGL-α or DAGL-β (2-AG)
- Degradation: FAAH or NAAA (AEA);
  MAGL, ABHD6, ABHD12, FAAH (2-AG)
- Oxidative: COX-2, LOX, CYP450

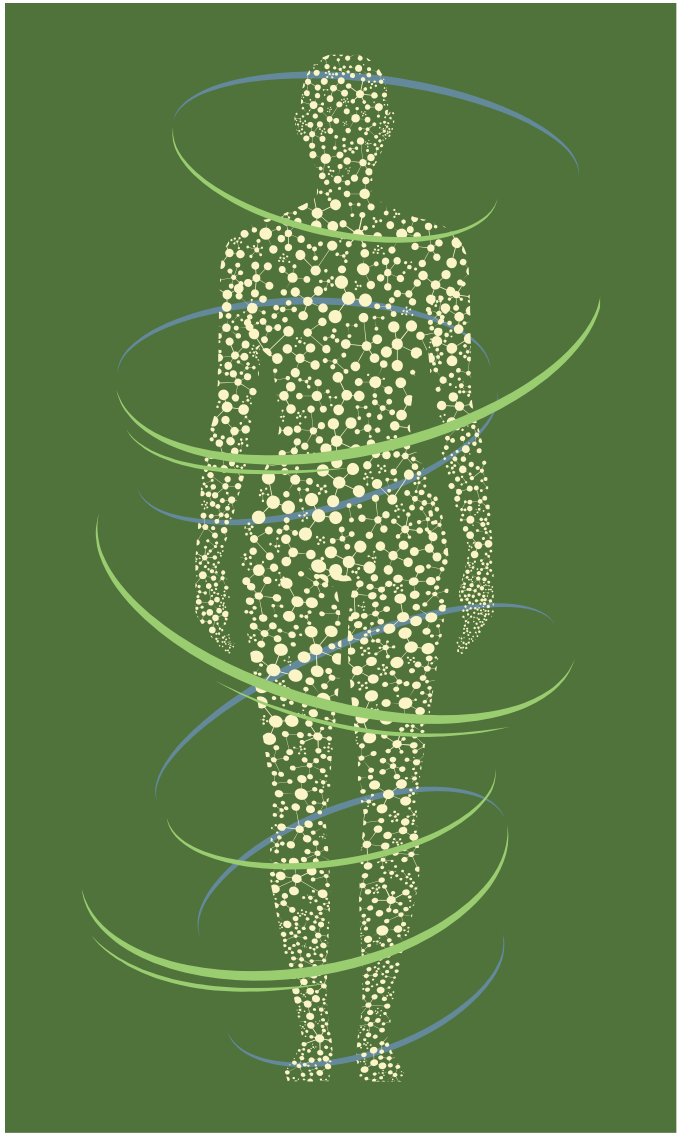

Image adapted from: Aizpurua-Olaizola et al. *Drug Discov Today*. 2017;22(1):105-110.

MAGL, monoacylglycerol lipase; NAAA, N-acylethanolamine acid amide hydrolase; NADA; *N*-arachidonoyldopamine; PPAR, peroxisome proliferator activated receptor; TRP, transient receptor potential (channel). $TRPV_1$, transient receptor potential vanilloid 1; COX-2, cyclooxygenase-2; LOX-lipoxygenase; CYP450, cytochromeP450; DAGL-α, diacylglycerol lipase alpha; DAGL-β, diacylglycerol lipase beta

Battista N et al. *Front Behav Neurosci*. 2012;6:9.
Aizpurua-Olaizola O et al. *Drug Discov Today*. 2017;22(1):105-110.

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Distribution of CB1 and CB2 Receptors

CB1 receptors

- CB1 receptors are the most abundant G-protein coupled receptors in the central nervous system (CNS) and are highly expressed in regions associated with cognition and movement[1]
- CB1 is also present in the peripheral nervous system and several peripheral organs[1]

CB2 receptors

- CB2 receptors are predominantly found in the periphery and are mainly involved in immune system functions[1]
- In the CNS, CB2 in microglial cells is upregulated in response to immune cell activation and neuroinflammation[2]

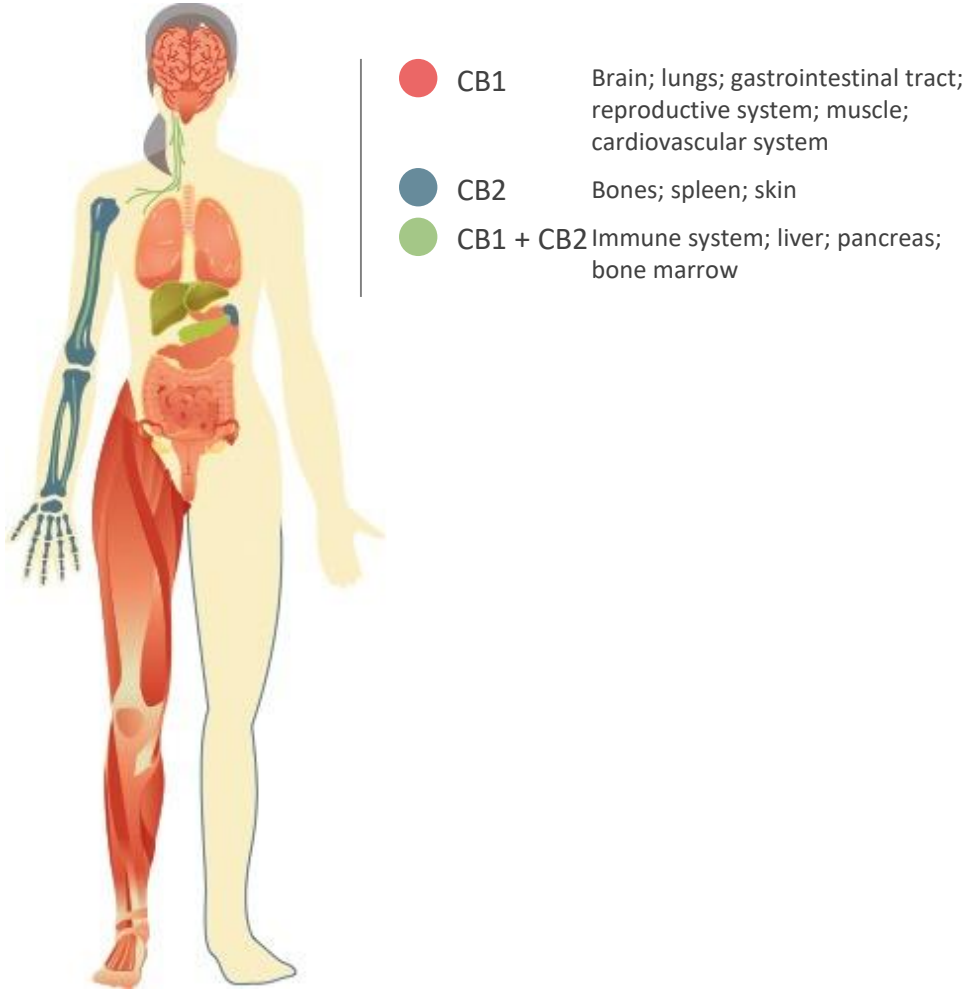

Image adapted from: Aizpurua-Olaizola et al. Drug Discov Today. 2017;22(1):105-110.

1. Kruk-Slomka M et al. *Mol Neurobiol*. 2017;54(10):8332-8347.
2. Stella N. *Neuropharmacology*. 2009;56(Suppl 1):244–253.

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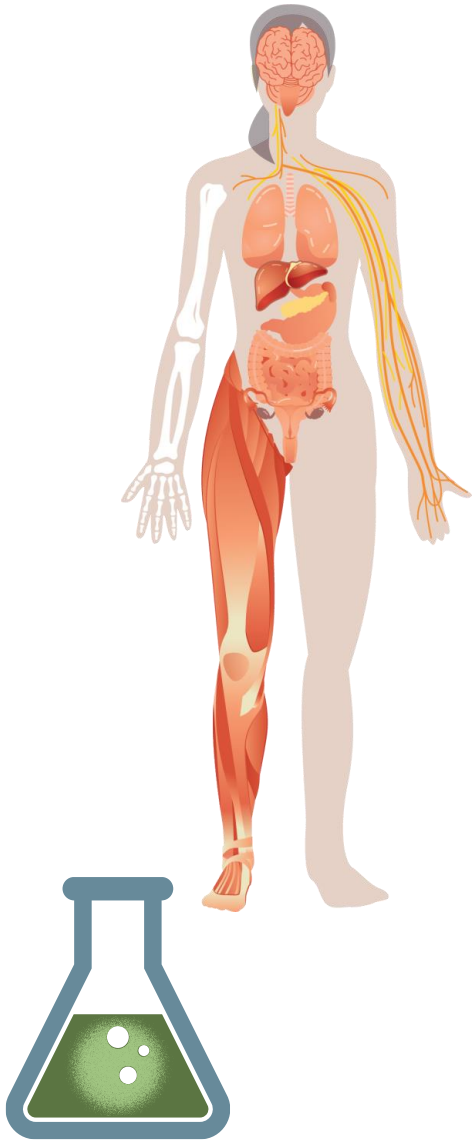
Endogenous and Exogenous Cannabinoids



Endogenous

- **Endocannabinoids and endocannabinoid-like compounds[1]**
  - Endogenous lipid mediators produced naturally in the body
  - 2-AG, AEA, NADA, PEA, OEA, virodhamine

Exogenous

- **Phytocannabinoids[2]**
  - Concentrated in the oily resin of the buds and leaves of plants such as *Cannabis* and *Helichrysum*
  - THC, CBD, CBG, CBDA, etc.
- **Synthetic cannabinoids[3]**
  - Manufactured by artificial means
  - Mimic the psychotropic effects of *Cannabis* but are associated with severe adverse effects

1. Battista N et al. *Front Behav Neurosci*. 2012;6:9.
2. Di Marzo V et al. *Neurotherapeutics*. 2015;12(4):692-698.
3. Cohen K et al. *Front Public Health*. 2018;6:162.

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The Body's Own Cannabinoids: **Endocannabinoids (eCBs)**

First known lipid-based neurotransmitters
Functionally different: regulation of food intake, immunomodulation, inflammation, analgesia, cancer, addictive behavior, epilepsy, and others

2-AG **AEA** **NADA**

virodhamine

Derived from arachidonic acid-containing phospholipids

Anandamide (AEA)

CNS: social behavior, stress response; periphery: pain

It degrades by the enzyme FAAH when no longer needed

2-AG (2-arachidonoylglycerol)

Many functions in CNS and periphery…

It degrades by the enzyme MAGL when no longer needed

Paloczi J et al. *Antioxid Redox Signal*. 2018;29(1):75-108.

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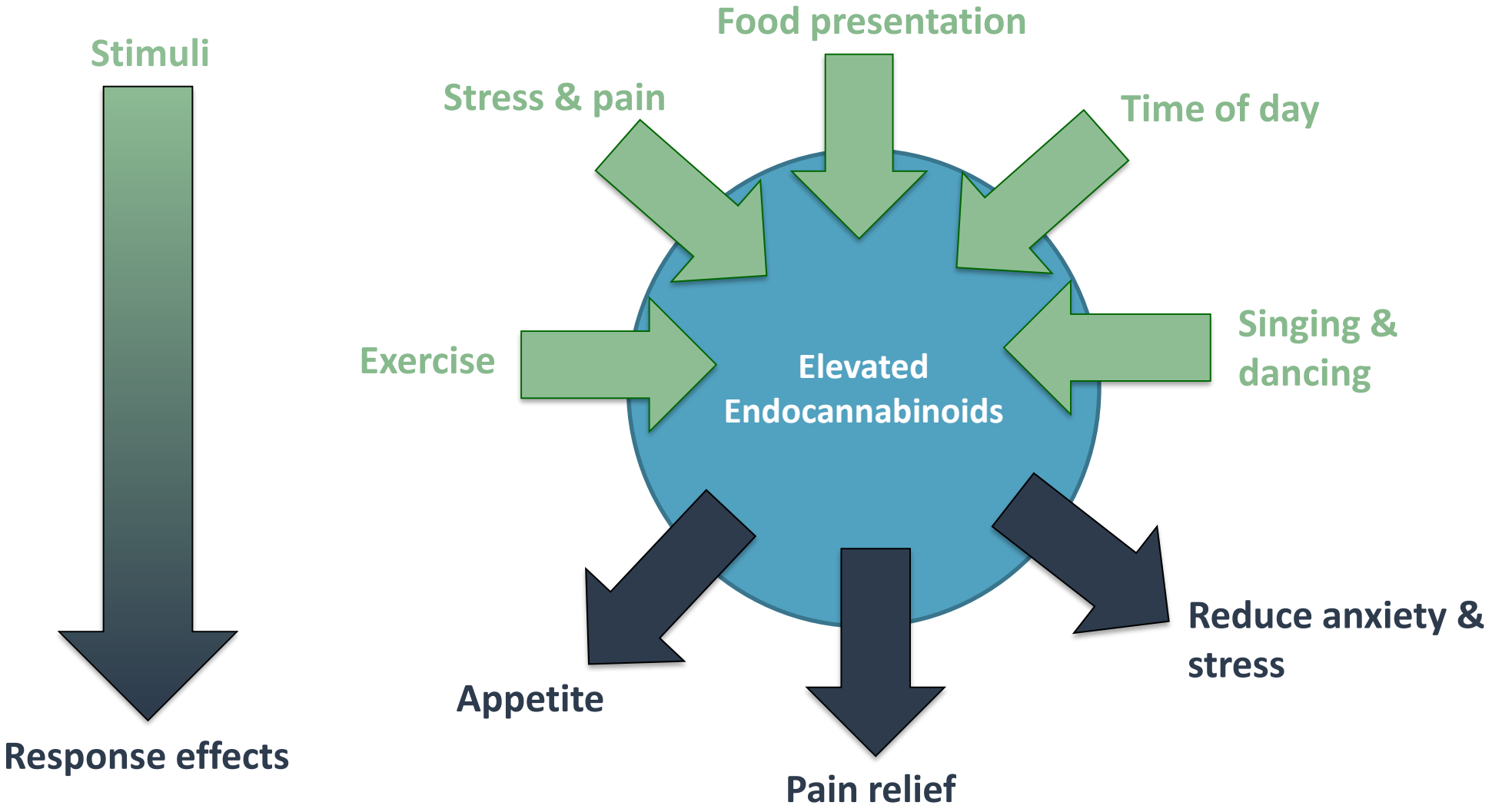
Factors Linked to Increased Endocannabinoids



Hillard CJ. *Neuropsychopharmacology Reviews.* 2018;43:155–172.

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What Is Endocannabinoid (eCB) Tone?

Humans have an underlying eCB tone that reflects the level of eCBs, their synthesis and catabolism, and cannabinoid receptor density

High tone
Obesity and associated disorders[2]

Low tone
Clinical endocannabinoid deficiency (CED)[1]

1. Russo EB. *Cannabis Cannabinoid Res*. 2016;1;154-165.
2. Richey JM et al. *Curr Diab Rep*. 2017;17(10):99.

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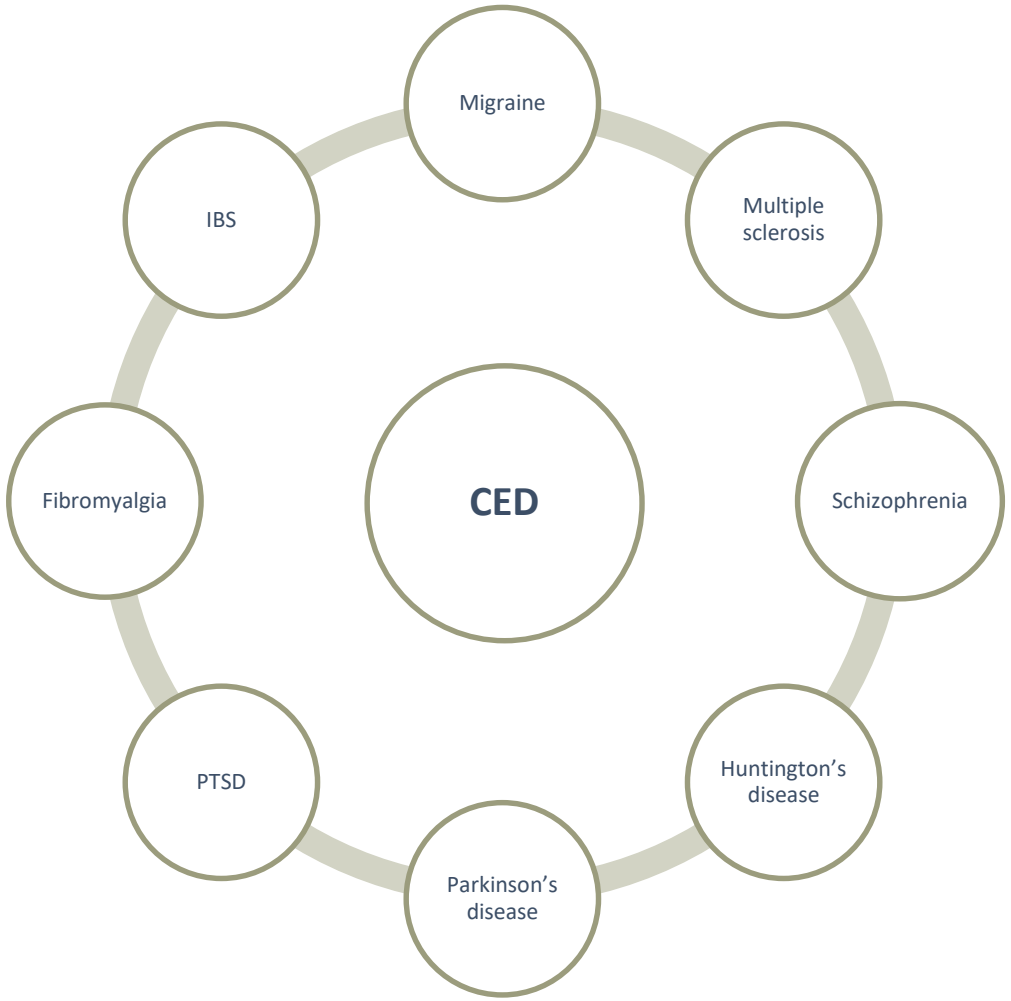
CED Is Central to Many Disorders

- CED may be genetic/congenital or acquired due to injury or disease
- Substantial objective evidence points to association with pathophysiological syndromes
  - Strongest evidence in migraine, fibromyalgia, and IBS
- Several strategies exist to rebalance the ECS



Russo EB. *Cannabis Cannabinoid Res*. 2016;1;154-165.
Leimuranta P et al. *Front Pharmacol*. 2018; 9: 420.

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High Endocannabinoid Tone and Development of Type 2 Diabetes

- Obesity increases eCB levels and/or CB1 receptor expression; high eCB tone contributes to further fat accumulation
- Independent of weight gain, high eCB tone:
  - Reduces insulin sensitivity in the liver, adipose tissue, and skeletal muscle
  - Causes loss of pancreatic β cells, leading to insulin deficiency

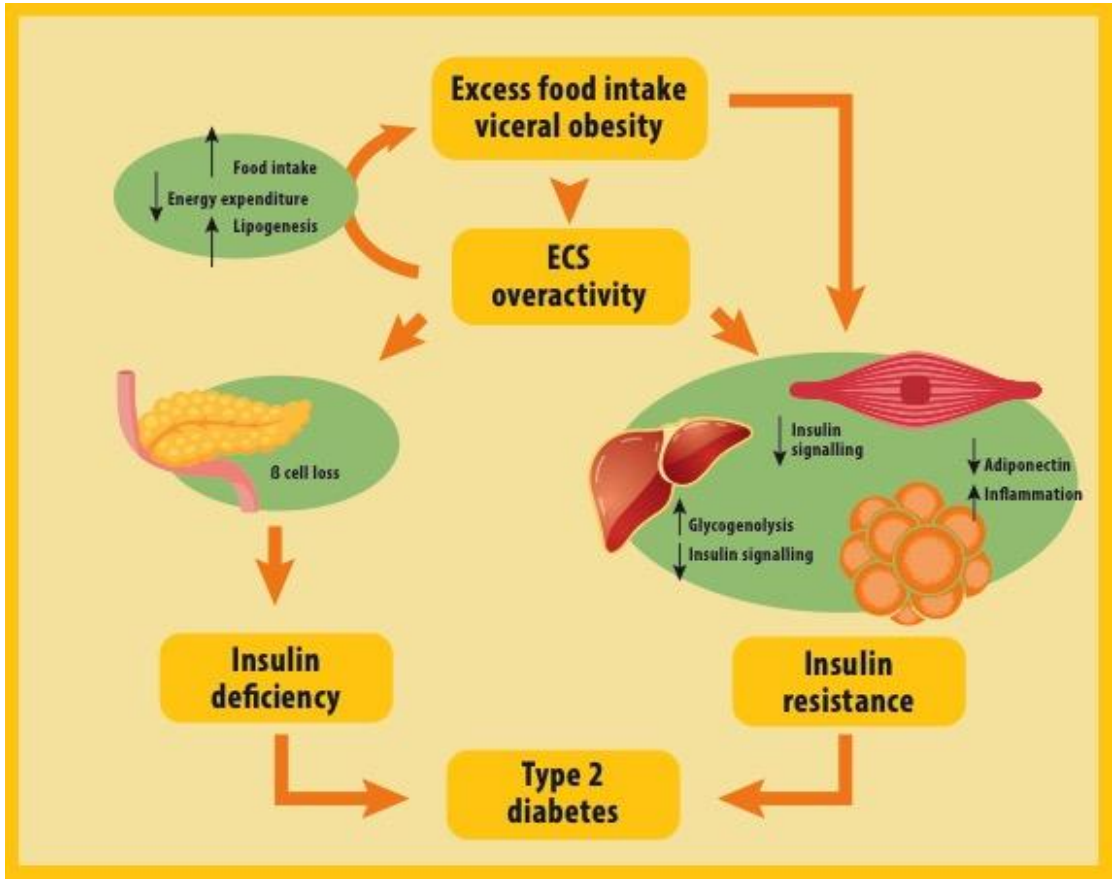

Figure adapted from: Gruden et al. *Br J Pharmacol*. 2016;173:1116-1127.

Gruden G et al. *Br J Pharmacol*. 2016;173:1116-1127.

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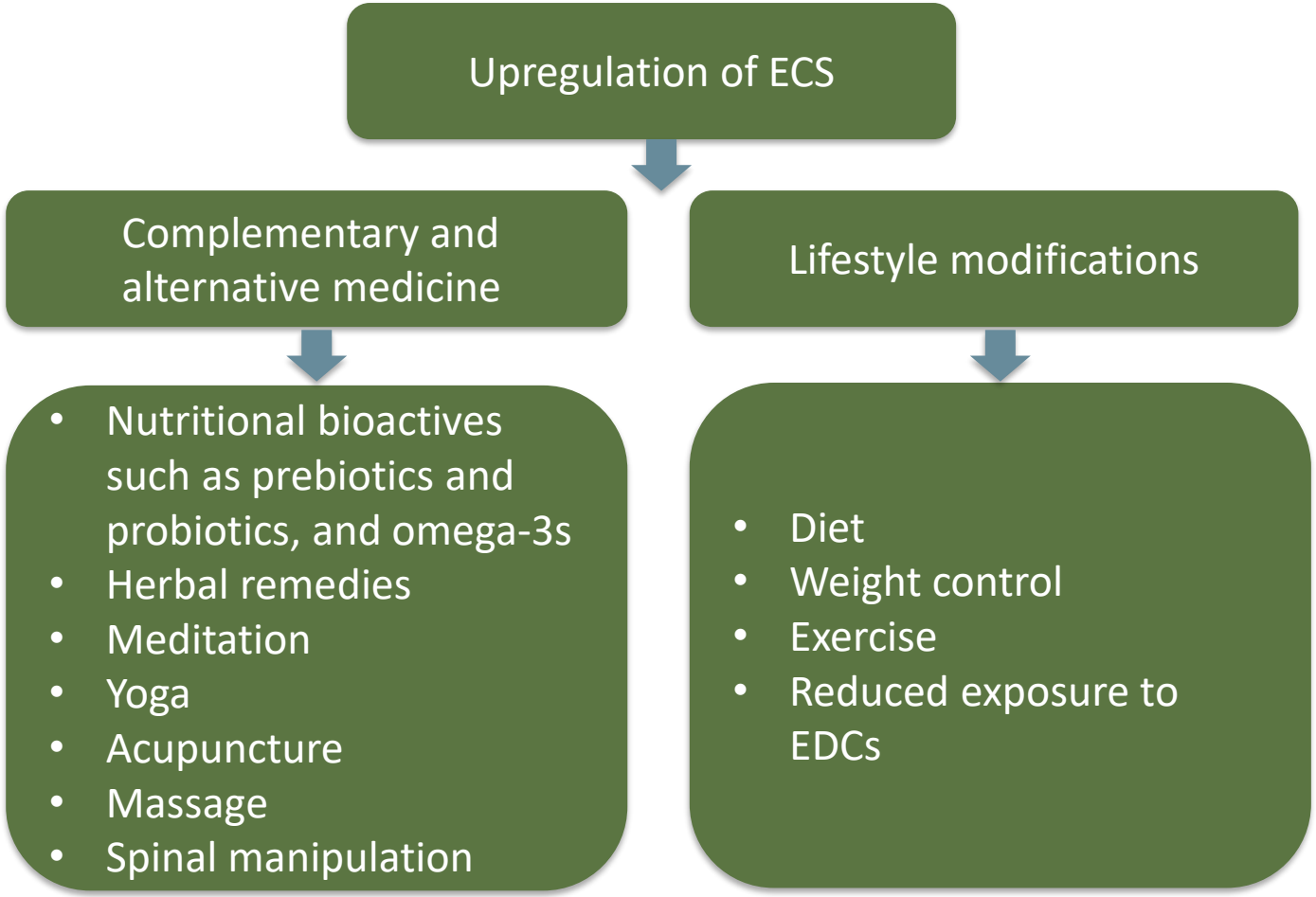
Potential Clinical Interventions for Supporting ECS Function



McPartland JM et al. *PLoS One*. 2014;9:e89566.

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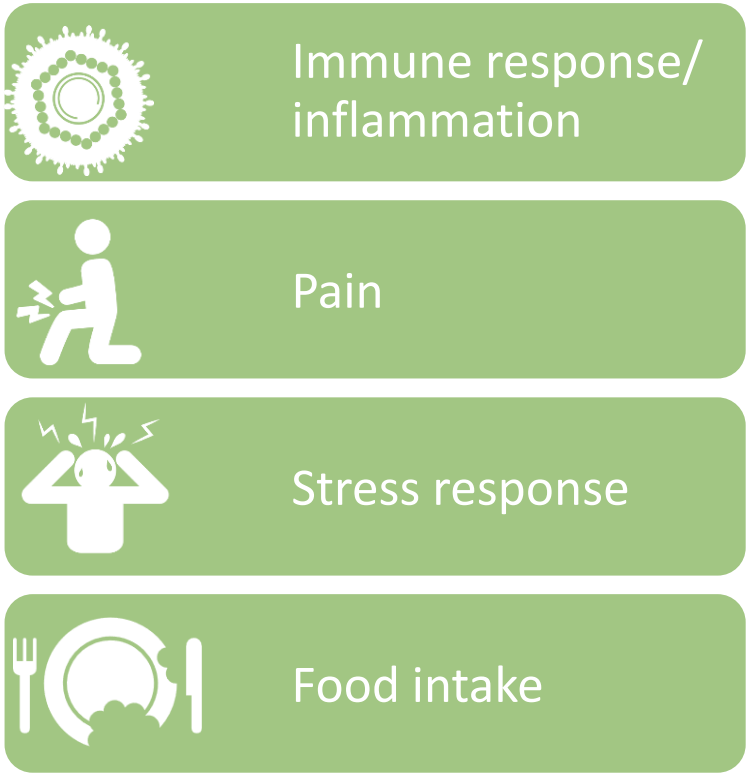
Summary: Key Points

- ECS is involved in regulating several physiological functions
- A balanced ECS is needed for optimal health
- eCB deficiency is evident in many disorders and can be modulated via several strategies, including lifestyle modification and administration of nutritional bioactives



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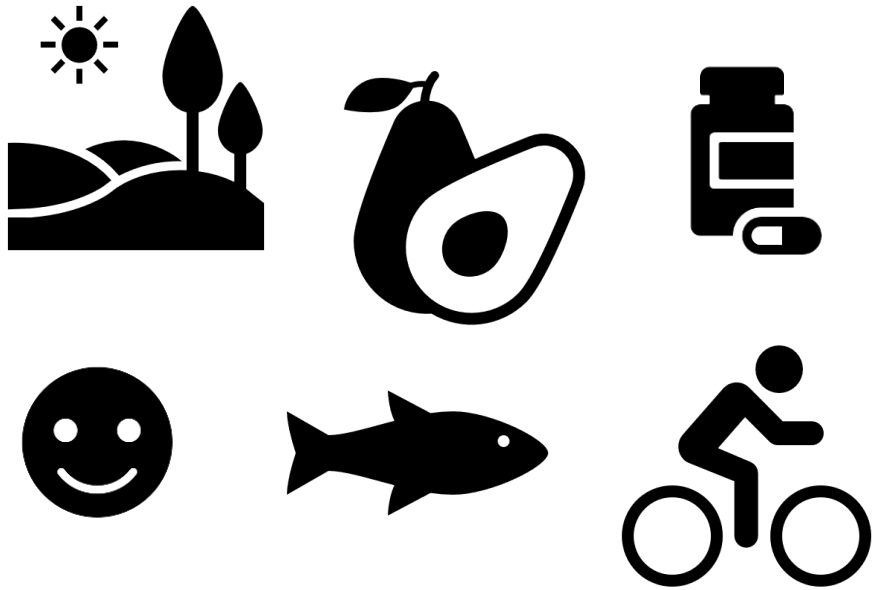

Imbalanced ECS tone **Balanced ECS tone**

Supporting and Nourishing the ECS with Nutritional Bioactives

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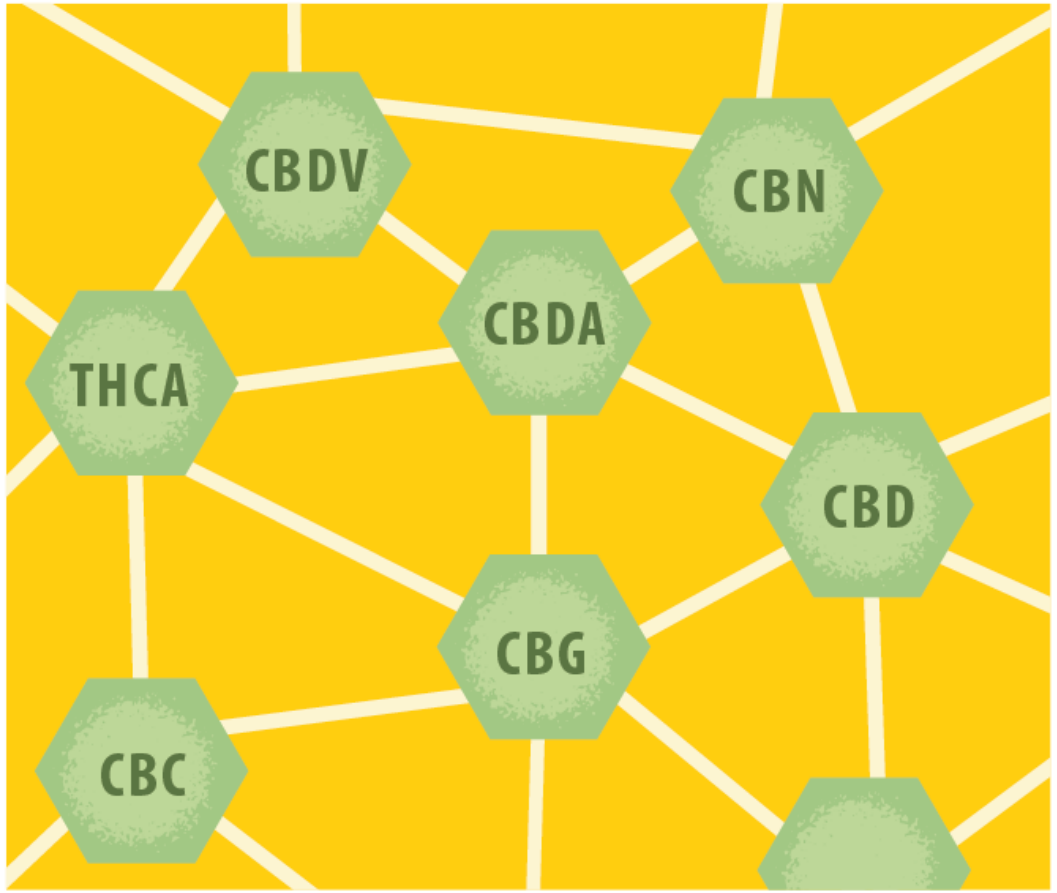
Phytocannabinoids

- Represent a group of plant-derived cannabinoids with largely produced in *Cannabis*[1]
- Predominant compounds found in *Cannabis,* THCA and CBDA, are pharmacologically inactive[1-2]
- THCA and CBDA are thermally unstable and can be decarboxylated when exposed to heat or light (smoking, cooking) into “active” phenolic THC and CBD[3]



CBD is the most prevalent nonpsychoactive cannabinoid in fiber-type *Cannabis* (hemp)[2]

CBDA, cannabidiolic acid; THCA, tetrahydrocannabinolic acid.

1. Andre CM et al. *Front Plant Sci*. 2016;7:19.
2. Citti C et al. *Front Plant Sci*. 2019;10:120.
3. Wang M et al. *Cannabis Cannabinoid Res*. 2016;1(1):262-271.

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Potential Therapeutic Uses of Phytocannabinoids

- Phytocannabinoids are naturally occurring cannabinoids that:[1,2]
  - Bind to cannabinoid receptors, triggering metabolic effects
  - Decrease the breakdown of endocannabinoids, increasing their availability
- These plant-derived compounds have potential for many therapeutic applications

CBD
- Antispasmodic
- Vasorelaxant
- Neuroprotective
- Antiepileptic
- Antisychotic
- Anxiolytic
- Immunosuppressive
- Anti-inflammatory
- Bone-stimulant
- Analgesic
- Intestinal anti-prokinetic
- Antipsoriatic
- Antidiabetic
- Antibacterial
- Antiemetic
- Antiproliferative anticancer
- Anti-ischemic

CBDV
- Bone-stimulant

Δ^9-THCA
- Antiproliferative
- Antispasmodic

Δ^9-THCV
- Antiepileptic
- Bone-stimulant
- Anorectic

CBG
- Bone-stimulant
- Antibactierial
- Antiproliferative

CBDA
- Analgesic
- Antiproliferative
- Anti-inflammatory

CBC
- Antiproliferative
- Antimicrobial
- Bone stimulant
- Anagesic
- Analgesic
- Anti-inflammatory

Adapted from: Izzo AA et al. *Trends Pharmacol Sci*. 2009;30:515-527.

1. Di Marzo V et al. *Neurotherapeutics*. 2015;12:692-698.
2. Izzo AA et al. *Trends Pharmacol Sci*. 2009;30:515-527.

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CBD has Multiple Mechanisms of Action

Binds cannabinoid receptors, CB1 and CB2, weakly
- Likely accounts for lack of psychoactivity

At low micromolar to sub-micromolar concentrations:
- Blocks equilibrative nucleoside transporter (ENT), GPR55, and the TRPM8 channel
- Enhances activity of:
  - 5-HT1a receptor
  - α1 and α1β glycine receptors
  - TRPA1 channel
- Has bidirectional effect on intracellular calcium

At higher micromolar concentrations:
- Activates the PPAR-γ and TRPV1 and TRPV2 channels
- Inhibits cellular uptake and FAAH-catalyzed degradation of anandamide

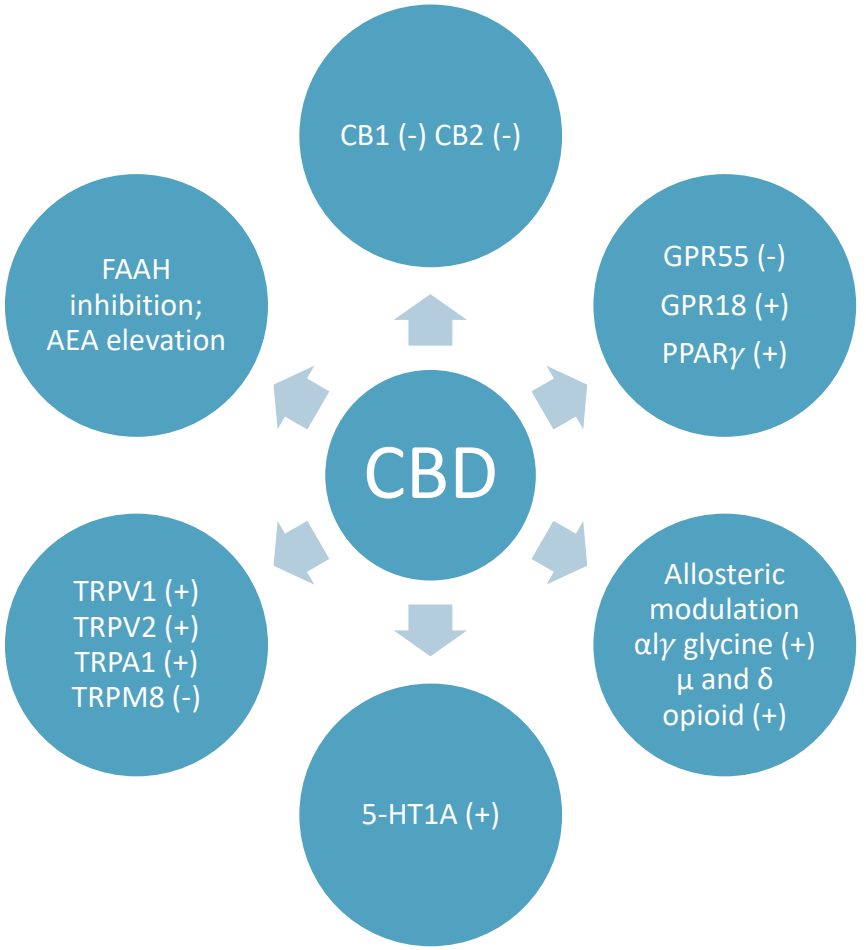

Figure adapted from: Massi P et al. *Br J Clin Pharmacol*. 2013;75(2):303-312.

Izzo AA et al. *Trends Pharmacol Sci.* 2009;30:515-527.

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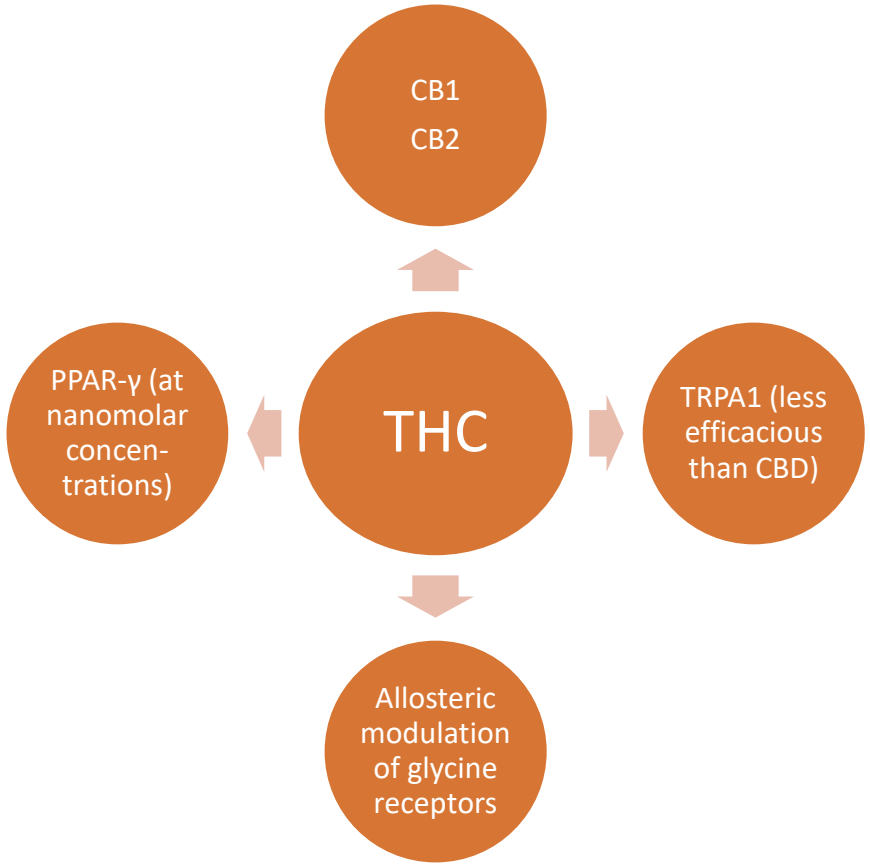
THC: Mechanisms of Action

- THC is the most psychotropic component in the *Cannabis* plant, and produces a wide range of psychoactive effects, such as feeling 'high', anxiety, paranoia, and cognitive deficits[1,2]
- By interacting with CB1 receptors, THC activates the brain's reward system, therefore, alters normal brain communication[1,2]
- Potential immunological or anti-inflammatory effects of THC are likely mediated via CB2 receptors[3]



1. Boggs D. et al. *Neuropsychopharmacology*. 2018;43(1):142–154.
2. Bloomfield M. et al. *Nature.* 2016;539(7629):369–377.
3. Pertwee RG. *Br J Pharmacol*. 2008;153(2):199-215.

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Interactions of CBD and THC and Their Effects on ECS

Preclinical studies

- CBD combined with isomeric tetrahydrocannabinols caused ‘synergistic hypnotic activity in the mouse’
- CBD inhibited THC effects on mouse catatonia, rat ambulation and rat aggression, but potentiated THC effects on mouse analgesia and rat rope climbing
- CBD decreased THC suppression of behavior in rats and pigeons
- CBD potentiated THC-induced changes in hepatic enzymes
- CBD increased THC potentiation of hexobarbitone in rats
- CBD increased THC reduction of intestinal motility in mice
- CBD reduced THC hypothermia and bradycardia
- CBD blocked THC inhibition of pig brain monamine oxidase
- CBD antagonized THC antinociceptive effects in mice
- CBD prevented tonic and clonic convulsions induced by THC
- CBD antagonized THC suppression of operant behavior in monkeys
- CBD delayed THC discriminative effects
- CBD prolonged THC cue effects in rats
- CBD antagonized THC catalepsy in mice
- CBD increased THC analgesic activity and anti-erythema
- CBD prolonged and reduced the hydroxylation of THC

Clinical studies

- CBD decreased anxiety and ‘psychotic scores’ caused by THC
- CBD slightly increased time to onset, intensity and duration of THC intoxication
- CBD attenuated THC euphoria
- CBD improved sleep and decreased epilepsy
- CBD decreased cortisol secretion and had sedative effects
- CBD provided antipsychotic benefits
- CBD attenuated the appetitive effects of THC

Adapted from: McPartland JM et al. *Br J Pharmacol.* 2015;172(3):737–753.

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Pharmacological Properties of Terpenoids

- β-caryophyllene is the only terpenoid that is able to bind to cannabinoid receptors
- However, all terpenoids interact synergistically with cannabinoids to produce physiological effects
- This is known as the **"entourage effect"**

Limonene	α-pinene	β-myrcene	Linalool
Antidepressant/ immunostimulant, anxiolytic, apoptosis of breast cancer cells, active against acne bacteria, dermatophytes, gastric reflux	Anti-inflammatory, bronchodilatory, acetylcholinesterase inhibitory	Inflammation blocking, analgesic, sedating, muscle relaxant, hypnotic, blocking of hepatic carcinogenesis by aflatoxin	Antianxiety, sedative, local anesthetic, analgesic, anticonvulsant/anti-glutamate
Also found in **lemon** 	Also found in **pine** 	Also found in **hops** 	Also found in **lavender** 
Nerolidol	**Phytol**	**β-caryophyllene**	**Caryophyllene oxide**
Sedative, skin penetrant, antimalarial, anti-leishmanial	Prevents vitamin A teratogenesis, GABA elevation	Anti-inflammatory, gastric cytoprotective, antimalarial, treatment of pruritus and addiction	Decreases platelet aggregation, antifungal, insecticidal/anti-feedant
Also found in **orange** 	Also found in **green tea** 	Also found in **black pepper** 	Also found in **lemon balm** 

Russo EB. *Br J Pharmacol*. 2011;163:1344-1364.

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Preclinical Evidence Supporting the Therapeutic Application of β-caryophyllene

Dementia[1-3]

- Neuroprotective effect
- Reductions in β-amyloid burden, microglial activation, COX-2 proinflammatory cytokines
- Improvement in cognitive deficits

Pain Management[4-6]

- Attenuation of mechanical allodynia
- Reduction in neuropathic pain
- Reduction in mechanical hyperalgesia, increase in muscle withdrawal thresholds

Metabolic Disorders[7-10]

- Alleviation of insulin resistance and oxidative-stress
- Restoration of antioxidant status, reduction in proinflammatory cytokines
- Promotion of glucose-stimulated insulin secretion
- Reduction in glucose, increase in insulin, restoration of carbohydrate metabolic enzymes

Depression & Anxiety[4,11-12]

- Antidepressant, anti-anxiety, anti-compulsive effects
- Reduction in depression-like behavior
- Anti-immobility effect

1. Hu Y et al. *Int Immunopharmacol*. 2017;51:91-98.
2. Lou J et al. *Front Pharmacol*. 2017;8:2.
3. Cheng Y et al. *Pharmacology*. 2014;94:1-12.
4. Aguilar-Ávila DS et al. *J Med Food*. 2019 Mar 13.
5. Segat GC et al. *Neuropharmacol*. 2017;125:207-219.
6. Quintans-Júnior LJ et al. *Life Sci*. 2016;149:34-41.
7. Youssef DA et al. *Biomed Pharmacother*. 2019;110:145-154.
8. Basha RH et al. *Clin Biol Interact*. 2016;245:50-58.
9. Suijun W et al. *Biochem Biophys Res Commun*. 2014;444:451-454.
10. Basha RH et al. *Acta Histochem*. 2014;116:1469-1479.
11. Bahi A et al. *Physiol Behav*. 2014;135:119-124.
12. de Oliveira DR et al. *CNS Neurol Disord Drug Targets*. 2018;17:309-320.

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What Is Hemp?

- Variety of *Cannabis* species
- Bred for seed, stalk (fiber and hurd), leaves, or flower
- Each component has many potential uses
- Contains nonpsychoactive phytocannabinoids
- Hemp is classified differently than marijuana

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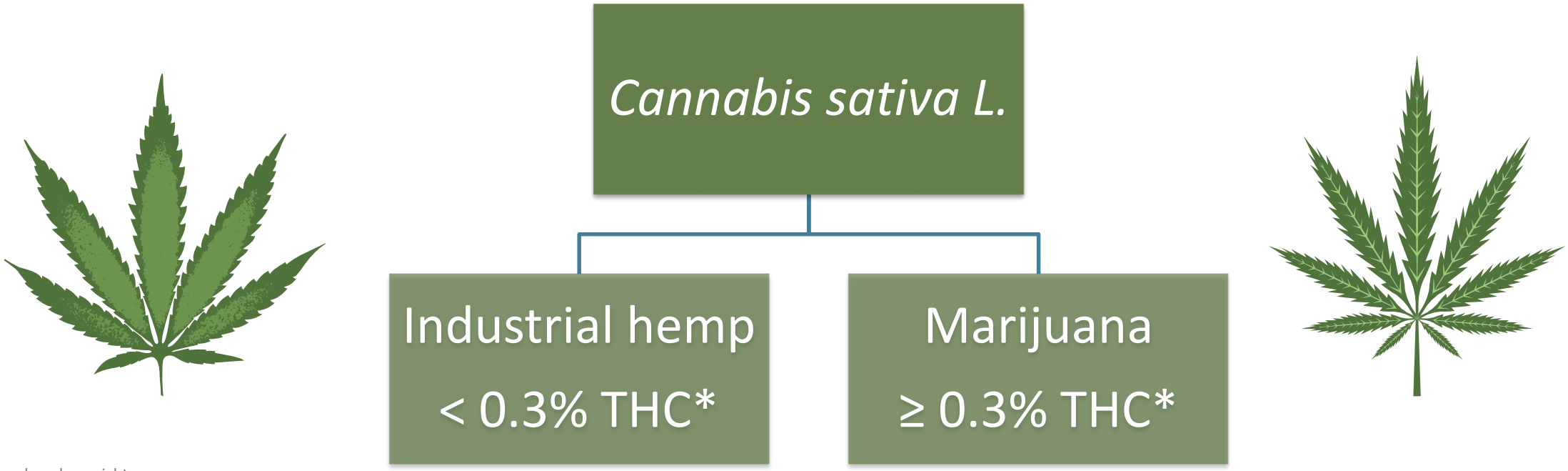
Hemp vs. Marijuana

- Cannabis classified as marijuana has a far higher concentration of THC relative to Cannabis classified of hemp; THC is the component of marijuana known for psychoactive effects
- Cannabis classified as hemp has a low concentration of THC; hemp is not known to have psychoactive effects



*Based on dry weight.

McPartland JM. *Cannabis Cannabinoid Res.* 2018;3(1):203-212.

NOTE: Definition of industrial hemp was created in Agricultural Act of 2014. https://www.congress.gov/bill/113th-congress/house-bill/2642. Accessed August 20, 2019.

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Hemp Extract Contains a Multitude of Bioactives (*in Addition to CBD*) that Modulate the ECS

Hemp extract

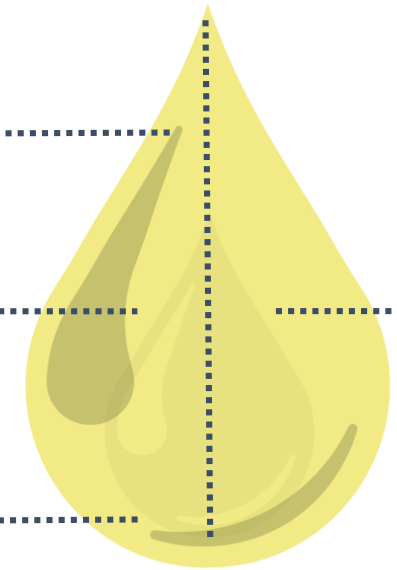
vs.

CBD isolate

Phytocannabinoids
e.g. CBD, CBDA, CBN, CBNA, CBG, CBGA

Terpenes
e.g. D-limonene, β-myrcene, β-caryophyllene

Phenolic compounds
e.g. flavonoids such as cannflavin A and B



Phytocannabinoids
CBD

CBDA, cannabidiolic acid; CBG, cannabigerol; CBGA, cannabigerolic acid; CBN, cannabinol; CBNA, cannabinolic acid.
Andre CM et al. *Front Plant Sci.* 2016;7:19.

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Improved Dose Response with CBD-Enriched *Cannabis* Extract

Preclinical study in mice with acute inflammation:

- Purified CBD (left) gives a bell-shaped dose response curve, which limits its potential clinical use
- By contrast, CBD-enriched *Cannabis* extract (right) shows a linear dose response
  - Higher doses are associated with increases in efficacy of anti-pain and anti-inflammatory responses

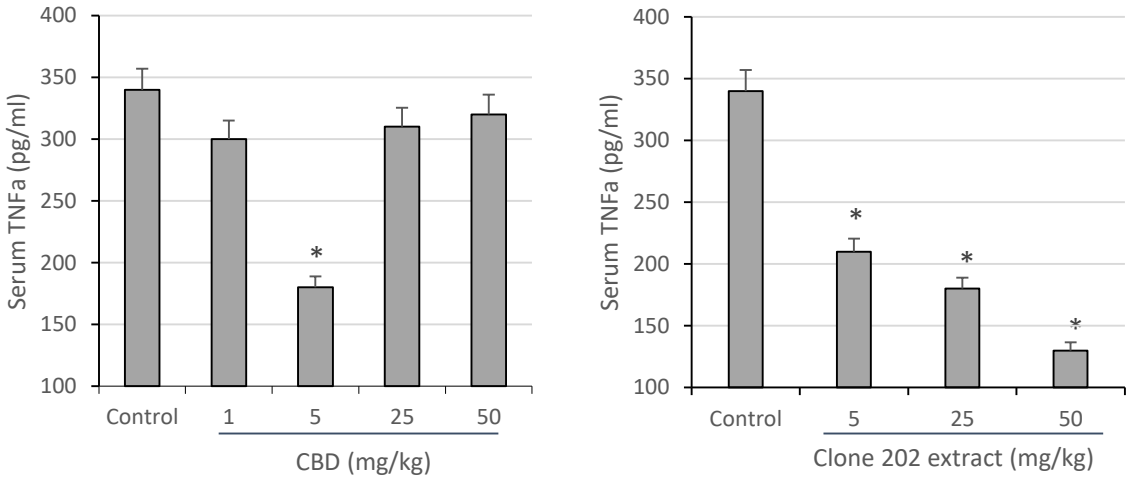

Figure adapted from: Gallily R et al. *Pharmacol Pharm*. 2015;6:75-85.

Gallily R et al. *Pharmacol Pharm.* 2015;6:75-85.

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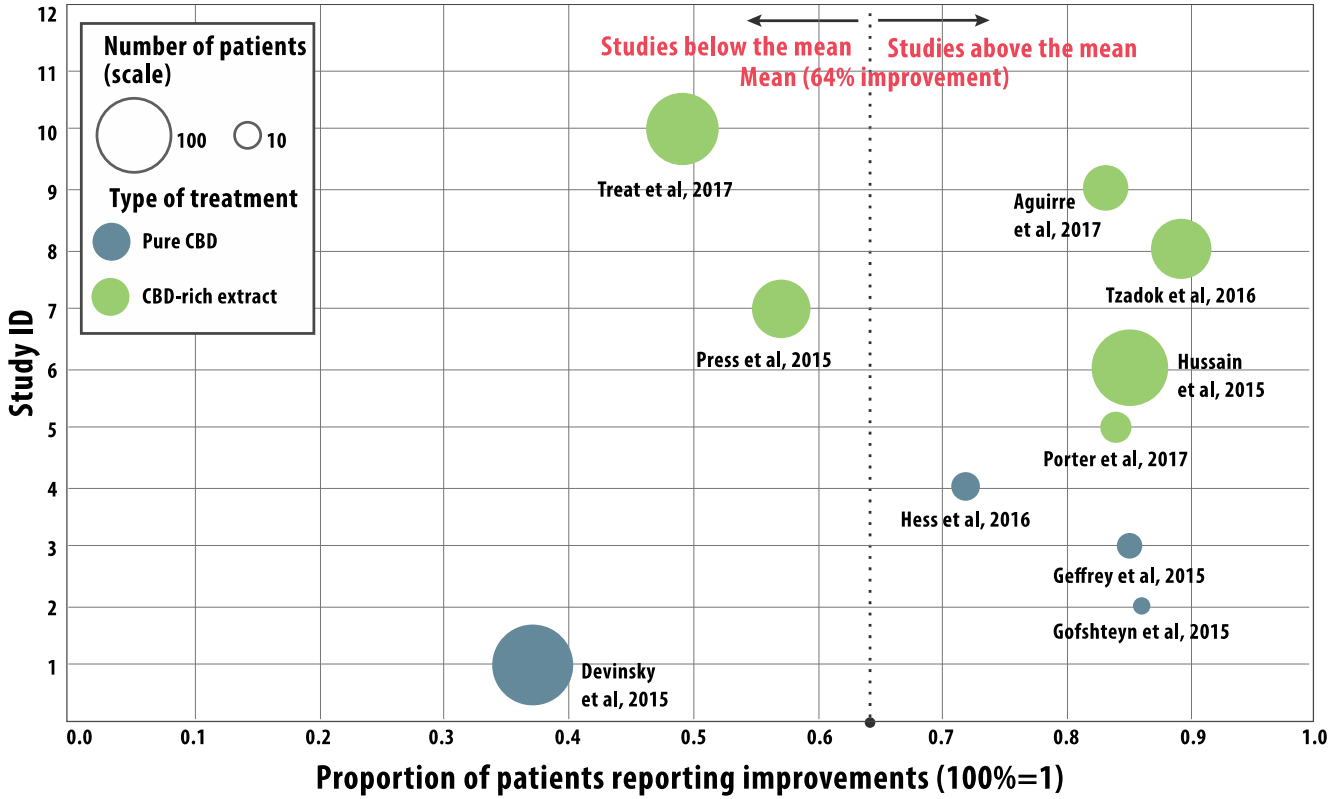
Clinical Benefits with CBD-rich *Cannabis* Extract

- This meta-analysis compared clinical effects of CBD-rich extracts to purified CBD in epilepsy
- Treatment with extracts was more likely to result in:
  - Improvements in seizure frequency in 2/3 of patients
  - Less reports of mild to severe adverse effects
  - Usage of lower average dose



The x axis represents the rate of clinical improvement (from 0 to 1, 100% = 1). The y axis is arbitrary "Study ID." The size of each point represents the number of patients included in the study and gives an idea of the "weight" of each study. The dotted line is the average, regardless of treatment.

Figure adapted from: Pamplona FA et al. *Front Neurol*. 2018;9:759.

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Pamplona FA et al. *Front Neurol*. 2018;9:759.

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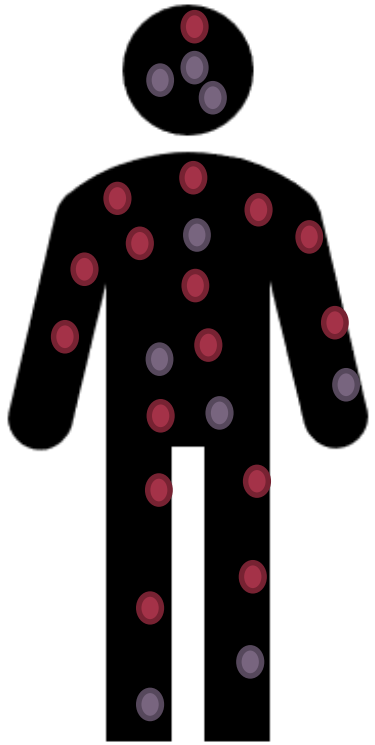
Binding to cannabinoid receptors, triggering metabolic effects



CB1/CB2 receptors

Non-CB receptors

Physiologic and metabolic effects

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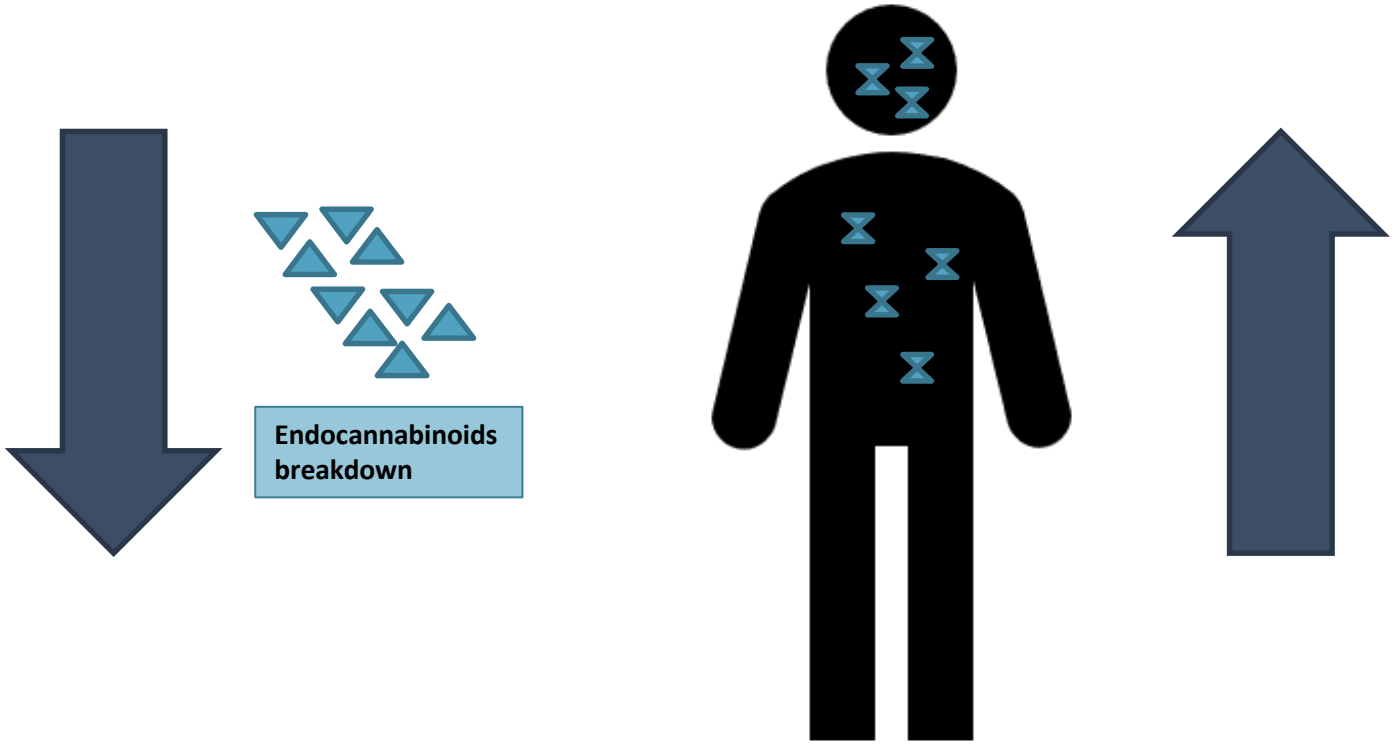
Decrease the breakdown of endocannabinoids, and their signaling termination; therefore, increasing their availability



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Safety Profile of Phytocannabinoids

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Safety of Phytocannabinoids

Chronic use and doses ≤ 1,500 mg/day of CBD reportedly well-tolerated in humans[1]

Nonpsychoactive

- Low concentration of THC (< 0.3%)[2]
- Phytocannabinoids with very low affinity for CB1 receptor[3]

Nonaddictive

- No tolerance develops with repeat dosing[4]

Pregnancy concerns: No hormonal or genotoxicity profiling

1. Iffland K et al. *Cannabis Cannabinoid Res*. 2017;2:139-154.
2. Holler JM et al. *J Anal Toxicol.* 2008;32:428-432.
3. Izzo AA et al. *Trends Pharmacol Sci.* 2009;30:515-527.
4. Hayakawa K et al. *Neuropharmacology*. 2007;52:1079-1087.

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Summary: Key Takeaways

- The endocannabinoid system (ECS) is a critical homeostatic regulator in the body
- The hemp plant contains several compounds that modulate the ECS and have many physiological benefits
- CBD is a phytocannabinoid that is often extracted, but hemp extract also contains multiple other active compounds and has therapeutic effects in many areas
- Terpenoids in hemp extract act synergistically with the phytocannabinoids, which widens the therapeutic possibilities of hemp extract

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