Cannabis in Veterinary Medicine: Cannabinoid Therapies for Animals

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Abstract

The use of cannabis for animal species is an area of growing interest, largely due to the therapeutic benefits being observed for humans and animals in the era of cannabis legalization. The close relationship humans have with their pets and other veterinary species has led to a renewed interest in the possibility and promise of cannabis to treat similar health issues in the animal community. This chapter explores the literature available on cannabis, its interactions with the endocannabinoid system, and how animal species interact with various formulations and cannabis treatments. A brief overview of the biology, chemistry, and history of cannabis is discussed with the relevance to veterinary species in mind. The pharmacologically active components are discussed with both anecdotal and objective, evidence-based, and clinical data.

Keywords

Cannabis · Cannabidiol · CBD · Cannabinoids · Nutritional supplement · Nutraceutical · Veterinary medicine · Animals · Veterinarian

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1 Introduction

The endocannabinoid system (ECS) has been identified in nearly all animals, from complex mammals like primates to phylogenetically primitive animals such as the cnidarians. The near universal presence and early emergence of the ECS, evolutionarily, is a strong indicator of its biological importance. Cannabinoid receptors are expressed in most animals, including vertebrates (mammals, birds, reptiles, and fish) and invertebrates (sea urchins, leeches, mussels, nematodes, and others). The most primitive animal an ECS has been observed in is the *Hydra* (*H. vulgaris*), a cnidarian in the class Hydrozoa, which is the first animal to develop a neural network. De Petrocellis et al. (1999) determined the major function of the ECS in the *Hydra* is to control the feeding response. It is evident from this data that all veterinary species contain an ECS. Therefore, an understanding of the ECS in these species is critical to the development of clinical applications for endocannabinoids and the phytocannabinoids derived primarily from *Cannabis sativa* L.

Clinical trials detailing the benefits and safety of phytocannabinoids in companion animals are finally being performed at academic institutions, after years of suppression of research due to the controversial status of cannabis. Public and commercial interest in this exciting and newly emerging therapy for animals has resulted in a number of studies and clinical trials being published or nearing publication. This chapter reports the findings of these studies that either have already been published, are in press, or are in their earlier stages; this chapter will provide personal communications with the principal investigators reporting on the results to date of their ongoing studies.

A thorough review of the literature reveals no published clinical trials prior to the studies reported in this chapter involving phytocannabinoids in dogs, cats, or horses. There are, however, quite a few studies in a laboratory setting using laboratory species to study the effects of phytocannabinoids, or to measure aspects of the ECS in a specific species.

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In spite of the paucity of published controlled studies in veterinary species, animal caregivers have been using cannabis for their dogs, cats, and horses since before the legalization of medical marijuana in 1996 and likely much earlier. Observed benefits from the use of cannabis include the reduction of anxiety; relief of pain; improvement of mobility in animals with osteoarthritis; reduction in tumor size; improved appetite; improved control of type 2 diabetes, inflammatory conditions, and digestive issues; and improved control of epileptic seizures. These benefits have not been universal, and successful treatments are based on dosages relative to the type of condition, severity of condition, size and metabolism, and factors related to biochemical diversity and density and distribution of the ECS among members of the same species.

Veterinarians, veterinary researchers, and animal caregivers are all eager to adopt cannabis-based therapies and have been looking for the evidence to support the safe and effective use of cannabinoid therapies for their pets or their patients. We will focus on providing that data in this chapter.

2 *Cannabis sativa* L.: Food, Herbal Medicine, Pharmaceutical, and Nutraceutical

Many of the constituents of *Cannabis sativa* can be classified as either a pharmaceutical ingredient, a nutrient, a nutraceutical, or an herbal product. Nutraceutical is a term that was created in 1989 by DeFelice by joining the two words, "nutrition" and "pharmaceutical" (Kalra 2003). It has no legal definition but refers to those compounds that are neither nutrients nor pharmaceuticals. The North American Veterinary Nutraceutical Council in 1996 defined a veterinary nutraceutical as a "[non-drug] substance which is produced in a purified or extracted form and administered orally to a patient to provide agents required for normal body structure and function and administered with the intent of improving the health and well-being of animals (Booth 2009)."

2.1 Regulatory and Legal Considerations

The Center for Veterinary Medicine of the Food and Drug Administration (FDA-CVM) states that the Dietary Supplement Health and Education Act (1994), which regulates dietary supplements for humans, does not pertain to animals. This means that legally there are only two choices for regulating products which are similar to human dietary supplements under the law; based on the intended use and contents, products are either regulated as animal food or animal drugs. There is no other choice. The marketing of these animal food/feeds or animal remedy products may also occur on a state-by-state basis and as such may be subjected to regulatory approval by local State Feed Control Officials (FCOs).

The Association of American Feed Control Officials (AAFCO) has no regulatory powers, but it provides guidelines for individual states regarding animal nutrition products. Their guidelines are followed in most states although interpretations, especially of allowable claims, vary from state to state and individual to individual. If a nutritional product contains ingredients that are not approved as food ingredients by the FDA-CVM, or the claims are violative, the local State FCO can recommend a product not be sold or actually issue a stop sale notice or confiscation of the product from the supplier and retail outlets.

In 2000, Iowa FCOs began to pull nutritional products that contained unapproved ingredients from the shelves of veterinary offices because they did not have FDA-CVM approval. At that time a trade group was formed, the National Animal Supplement Council (www.NASC.cc) to work with the regulators at both the state and federal levels to develop workable guidelines for suppliers to follow that would satisfy the regulators' concerns about these products that were commonly referred to as nutraceuticals.

As a result of NASC's efforts, both products for health purposes are referred to as "dosage-form animal health products" and may be marketed under enforcement discretion provided companies act responsibly. The guidelines for company responsible behavior include but are not limited to labeling products properly; making claims that are not in violation of Sect. 201(g)(1)(b) of the Federal Food, Drug, and Cosmetic Act (FFDCA); following Good Manufacturing Practice Standards and implementing effective risk monitoring/management systems; ensuring product safety; and protecting both the health of animals and people which are always the most important consideration for any regulatory official (Fig. 1).

The FDA-CVM developed confidence in the work of the NASC when this trade group established a website for

Fig. 1 *NASC is an organization that* regulates the quality of animal-based nutritional supplements

veterinary nutraceutical adverse event reporting. This database, NAERS™, now contains risk management data, product labels, and statistical analysis on hundreds of nutraceutical and nutritional products and ingredients. NAERS contains many millions of recorded administrations and can accurately give the percent and type of adverse events recorded for a given material over this large number of animal administrations. The NASC Adverse Event Reporting System (NAERS™) is currently the most advanced data base in the world for dosage-form products for dogs, cats, and horses.

The NASC started with 18 members in 2001 and as of 2018 now has well over 175 members. Member companies must comply with cGMP standards, follow label template guidelines, and not make medical claims, but can use structure and function statements in their marketing materials and labels. Members must undergo regular audits by a third party that include random testing of their products to verify they meet label claims. Member companies who pass the audit are allowed to display the NASC seal on their bottles, or other marketing materials, which, for the consumer, has become a sign of quality, and many pet owners will select only those products that display this seal.

The FDA-CVM tightly regulates what ingredients are approved for animal feed. Hemp is currently not an approved ingredient for animal feed according to the FDA-CVM and the AAFCO (Association of American Feed Control Officials; www.AAFCO.org). Additionally, according to the FDA, in order for a material to be considered a supplement (for humans) versus a pharmaceutical, it needs to have been in use as a supplement prior to October 1994 when DSHEA was passed, or the company must file an NDI (New Dietary Ingredient) application. Currently, the FDA claims that there was no recognized use of hemp as a nutraceutical before GW Pharmaceuticals filed their IND for Epidiolex, their recently FDA-approved, CBD-containing drug to treat resistant pediatric epilepsy due to Dravet's syndrome. The limits surrounding this issue are currently being evaluated and will likely be argued in court.

The Drug Enforcement Agency has persisted in its controlled substance classification of the non-THC resins found in the cannabis plant, whether it's from the state-legal medical cannabis with high THC (aka: marijuana) or the federally legal low THC (aka: hemp). This is in spite of the fact that non-THC phytocannabinoids meet none of the requirements for a controlled substance that is a Schedule I. This scheduling category states that materials from this category have no medical applications and a great potential for abuse and/or toxicity. This Schedule I classification is in spite of the fact that the Federal Government holds a patent for the medicinal applications of CBD for neuroprotection (Hampson et al. 2003; US Patent #6630507). This classification of the non-THC resins in the hemp as a Schedule I plant needs to be changed to a nonscheduled classification in order for the nascent and burgeoning hemp industry to fully develop economically. As of Summer 2018, there is a bill introduced into Congress to remove the controlled substance status for hemp and all of its derivatives as a part of the 2018 Farm Bill.

The FDA-CVM, in a public statement in April 2018 about the medical use of cannabis in animals, points to the fact that currently there are no FDA-approved drugs for animals that contain cannabis, and there is no scientific evidence, as of Spring 2018, that supports the use of cannabis and/or CBD in animals. However, with a number of university veterinary studies now showing both safety and efficacy, that may change. The FDA has now approved Epidiolex™ as of June 2018 but has yet to be rescheduled by the DEA. The use of the term "CBD" or "cannabidiol" on a label or in the marketing of a hemp product for people or pets now has become a medical claim since CBD is in an approved drug, and this may leave the product open to enforcement by the FDA. Companies need to avoid using these two words and must choose other language when describing the contents of a product and its intended activity to the end user. Currently there is no established limit for naturally occurring CBD in hemp products, although it has been considered by regulatory agencies to keep the CBD content at or below that which is naturally occurring in the hemp plant, which in some cultivars may average 18% in the flowers. This potency limit has not yet been established, though.

The 2018 US Farm Bill and the Controlled Substance Act of 1970

Sect. 7606 of the 2013 US Farm Bill allowed for research into hemp cultivation and market research into the commercialization of hemp on a state-by-state basis, for each state that has passed legislation regarding hemp cultivation and commercialization in that state. Currently there are 34 states that have legislation on the books allowing for hemp cultivation, with Kentucky and Colorado the largest producers.

The 2018 Farm Bill contains the McConnell amendment which removes the controlled substance status of legally grown hemp, again, on a state-by-state basis. Each state can decide how they choose to regulate hemp within their state according to this amendment. Once the Farm Bill is passed with this amendment, it will remove any legal impediment to the commercialization of hemp and hemp derivatives in the United States.

This amendment is necessary because the Drug Enforcement Administration (DEA) has scheduled all cannabis (both hemp and marijuana) as a Schedule I controlled substance, which means it has no medical applications and is potentially toxic and/or addictive. Controlled substances cannot be transported across state lines, so many companies (especially the larger ones) that would normally be marketing a popular supplement like CBD will not get involved with commercializing hemp until this last barrier to trade is removed.

2.2 Hemp in Animal Feed

Hemp is becoming an agricultural commodity again in the United States, as the stigmas and restrictions on research and commercialization are gradually being removed as a result of the US Farm Bill of 2013, Section 7606. This landmark *omnibus* spending bill established the legal status of growing hemp in the United States, and as a result, an interest in the use of hemp for animal feed has developed.

The Colorado legislature passed a bill forming a stakeholders committee under the guidance of the Colorado Department of Agriculture to examine the use of hemp and hemp derivatives for animal feed for both companion animals and food animals. The final report of that stakeholders commission was published on December 29, 2017 (Glenn 2017).

To Summarize the Findings of This Stakeholders' Committee Report

The FDA and AAFCO both stated they would be receptive to a Feed Additive Petition (FAP) submitted to the FDA for the use of the noncontrolled substance part of the plant, the grain or sterilized seed, including the oil and its protein cake. This FDA approval is sought for dogs, cats, horses, and food animals. In animals destined to enter the human food chain, there will need to be studies demonstrating conclusively the safety to our food supply to feed a food that contains cannabinoids, even trace amounts of the non-psychoactive phytocannabinoids.

The Hemp Industries Association of Colorado has formed a steering committee to create a Feed Additive Petition for Hemp Seed to submit to the FDA-CVM for approval of hemp seed as an animal feed ingredient. Currently, hemp is an unapproved nutritional ingredient according to the FDA, and as such when contained in animal feed is considered to be adulterated, and may be subject to regulatory action when marketed as a food. Following a literature review, 6-month safety feeding studies will need to be performed before the FDA would agree to approve hemp as a feed ingredient. This process usually takes 2–3 years and a considerable amount of resources.

Currently, any animal food products that contain hemp that are labeled as nutritional products with a guaranteed analysis and hemp labeled as a nutrient will be considered adulterated until hemp is an approved feed ingredient. For now, hemp will have to be included in a product as a nutraceutical and have the label language reflect the support that hemp affords to the healthy structure and function of the body and be marketed as a dosage-form animal health product. This is provided the product meets the minimum limits for THC and does not contain phytocannabinoid concentrations that are greater than what would be normally found in the whole hemp plant on average.

The Nutritional Value of *Cannabis sativa* L.

Cannabis seed, also known as "grain," contains both valuable fatty acids and high-quality protein. The seed is particularly nutritious and is often consumed whole or used in food preparations. A protein cake made from the seed has been used for animal feed in Europe. Whole hemp seed contains approximately 20–25% protein, 20–30% carbohydrates, and 10–15% insoluble fiber (Callaway 2004; Deferne and Pate 1996). In addition, it contains a mixture of the saturated fatty acids palmitic and stearic acid as well as oleic acid. Hemp seed oil is an extremely rich source of unsaturated fatty acids, especially the essential fatty acids linoleic acid (LA) and alpha-linolenic acid (ALA) (Callaway 2004; Leizer et al. 2000).

Essential fatty acids (EFAs) cannot be produced naturally by an animal's body and must be sourced from the diet. LA and ALA are omega-6 and omega-3 fatty acids, respectively, and, in the dog, are considered to be essential, since the dog cannot synthesize them. In cats these two fatty acids are also essential; however, due to their liver function, cats cannot desaturate and elongate linoleic acid to form arachidonic acid (Hand et al. 2010).

Hemp seed oil also tends to contain high amounts of gamma-linolenic acid (GLA) and stearidonic acid (SDA), which are metabolites of LA and ALA (Callaway 2004). Since these metabolites are produced by the breakdown of dietary LA and ALA, they are not considered EFAs. However, supplementation in the diet can be extremely beneficial. Many chronic diseases of modern society, including cancer, diabetes, heart disease, arthritis, atopy, Alzheimer's disease, and others, have an inflammatory component (Kapoor and Huang 2006). Diets enriched in GLA have been shown to reduce inflammation (Tate et al. 1989), and therefore the nutritional value of GLA from hempseed oil is clear.

Cannabis seed oil has the proper ratio (4:1) of omega-6 to omega-3 fatty acids for optimal health. The pro-inflammatory eicosanoid cascade is promoted by a high omega-6/omega-3 fatty acid ratio. This results in increased *prostaglandin* 2a ($PG_2\alpha$), which is a pro-inflammatory eicosanoid. Diets rich in omega-3 s and poor in omega-6 s have a less pro-inflammatory nutrient profile, which can be anti-inflammatory with sufficiently high levels of long-chain polyunsaturated omega-3 fatty acids (LCPUFA) and/or the anti-inflammatory omega-6 LCPUFA, gamma-linolenic acid (GLA). The Western diet, high in grain-fed meat and grains which are all high in omega-6 fatty acids and low in omega-3 fatty acids, results in a 20:1 ratio of omega-6/omega-3. It is this high omega-6/omega-3 ratio that shunts the eicosanoid cascade to the pro-inflammatory side and which dietary omega-6 fatty acids and GLA will serve to reduce over inflammatory trends in the body (Bauer 2011).

Three clinical examples where the lower omega-6/omega-3 ratio tends to a less inflammatory environment in the body

are the following: (1) a ratio of 4:1 omega-6/omega-3 was associated with a 70% decrease in total mortality from cardiovascular disease in human patients; (2) in colorectal cancer a decrease in rectal cell proliferation was observed with a lower ratio of 2.5:1; and (3) a ratio of 5:1 was found beneficial in asthma patients (Simopoulos 2002).

3 Modern History of Cannabinoid Pharmacology

The therapeutic properties of cannabis have been appreciated over several millennia and its pharmacological properties studied since the mid-1900s. This effort was enhanced in the 1960s with the isolation, characterization, and subsequent synthesis of its major psychoactive ingredient (−)-Δ^9-THC by Mechoulam and Gaoni, and significant attention was given to developing synthetic compounds with more potent and targeted therapeutic effects (Gaoni and Mechoulam 1964). Concurrent approaches by individual research laboratories and the pharmaceutical industry produced a series of structurally related new compounds, which were collectively called cannabinoids. One of these drug development projects by Eli Lilly led to the first synthetic cannabinoid, nabilone (Cesamet), which was used to treat nausea, pain, and reduced appetite associated with cancer chemotherapy (Makriyannis 2014; Pertwee 2008). The major breakthrough in understanding the mechanism of action of cannabis, and more specifically Δ^9-THC, came with the discovery of the first biological targets for this compound. Following a very successful research meeting in 1987 by the National Institute on Drug Abuse in Bethesda, Maryland, collaborative work among the participating laboratories led to the discovery of a new G-protein-coupled receptor (GPCR), which was named CB_1, to be followed 2 years later by a second GPCR named CB_2 (Makriyannis 2014). The CB_2 cannabinoid receptor is mostly found in immunity-related cells, and it is largely involved in regulating the immune system and inflammatory conditions. Although under normal "homeostatic" conditions the CB_2 receptors have a very low presence in neurons, in "non-homeostatic" situations such as inflammation, neurodegenerative diseases such as Alzheimer, Parkinson, ALS, as well as in cancers such as gliomas, its presence in the brain is dramatically increased in astrocytes as well as microglial and cerebromicrovascular endothelial cells. Recent published work has provided evidence that this receptor may also have a role in modulating addictive disorders (Xi et al. 2011).

Both the CB_1 and CB_2 receptors play important roles in many processes including neuronal plasticity, pain, anxiety, neuroinflammation, immune function, metabolic regulation, reward, craving, and bone growth (Mackie 2006).

4 Endocannabinoid (eCB) Chemistry

The next discovery on the ECS was a group of compounds found in mammalian tissues, which were named endocannabinoids (eCBs). The most representative of which were arachidonoyl ethanolamide or anandamide (AEA, circa 1992), a long-chain fatty acid amide, and 2-arachidonoyl glycerol (2-AG circa 1995), the respective ester (Devane et al. 1992; Mechoulam et al. 1995; Pertwee 2000). These new substances are capable of activating both CB_1 and CB_2 receptors and, when tested in animals, produced biological effects paralleling those of Δ^9-THC (Fig. 2).

eCBs are produced on demand by a series of enzymes that are present within the cell membrane and are activated by elevated levels of calcium ions. eCB levels, also referred to as the "endocannabinoid tone," are tissue dependent and are regulated by another set of enzymes, the most prominent of which are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), whose role is to deactivate AEA and 2-AG and their congeners, respectively. An additional feature in the regulation of eCB tone is a transport mechanism that is involved in transporting released eCBs into the cell (Fig. 3) (Vemuri and Makriyannis 2015).

Anandamide is synthesized from its membrane precursor *N*-arachidonyl phosphatidylethanolamine (NAPE) through cleavage by a phospholipase D (NAPE-PLD). In contrast, anandamide is degraded by the enzyme fatty acid amide hydrolase (FAAH) into arachidonic acid (AA) and ethanolamine. 2-AG is produced through the activities of either diacylglycerol lipase (DAGL) or phospholipase C (PLC) using an AA precursor. 2-AG is then subsequently degraded by monoacylglycerol lipase (MAGL) into glycerol and AA (Hartsel et al. 2016)

eCBs are released from the postsynaptic neuron and act on cannabinoid receptors on the presynaptic neuron. The eCBs inhibit the influx of calcium intracellularly resulting in inhibition of neurotransmitter release. The actions of eCBs are relatively short lived as they undergo rapid reuptake by the cell and are then degraded. The production of eCBs can be

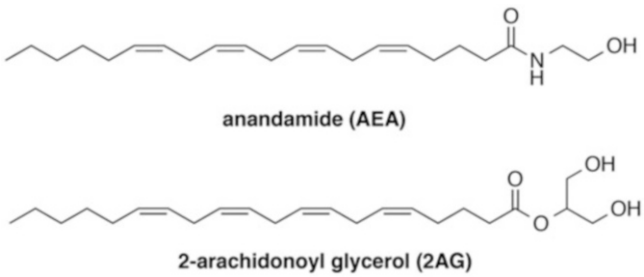

Fig. 2 Endocannabinoids AEA and 2-AG bind to the CB receptors that are endogenously produced inside the body

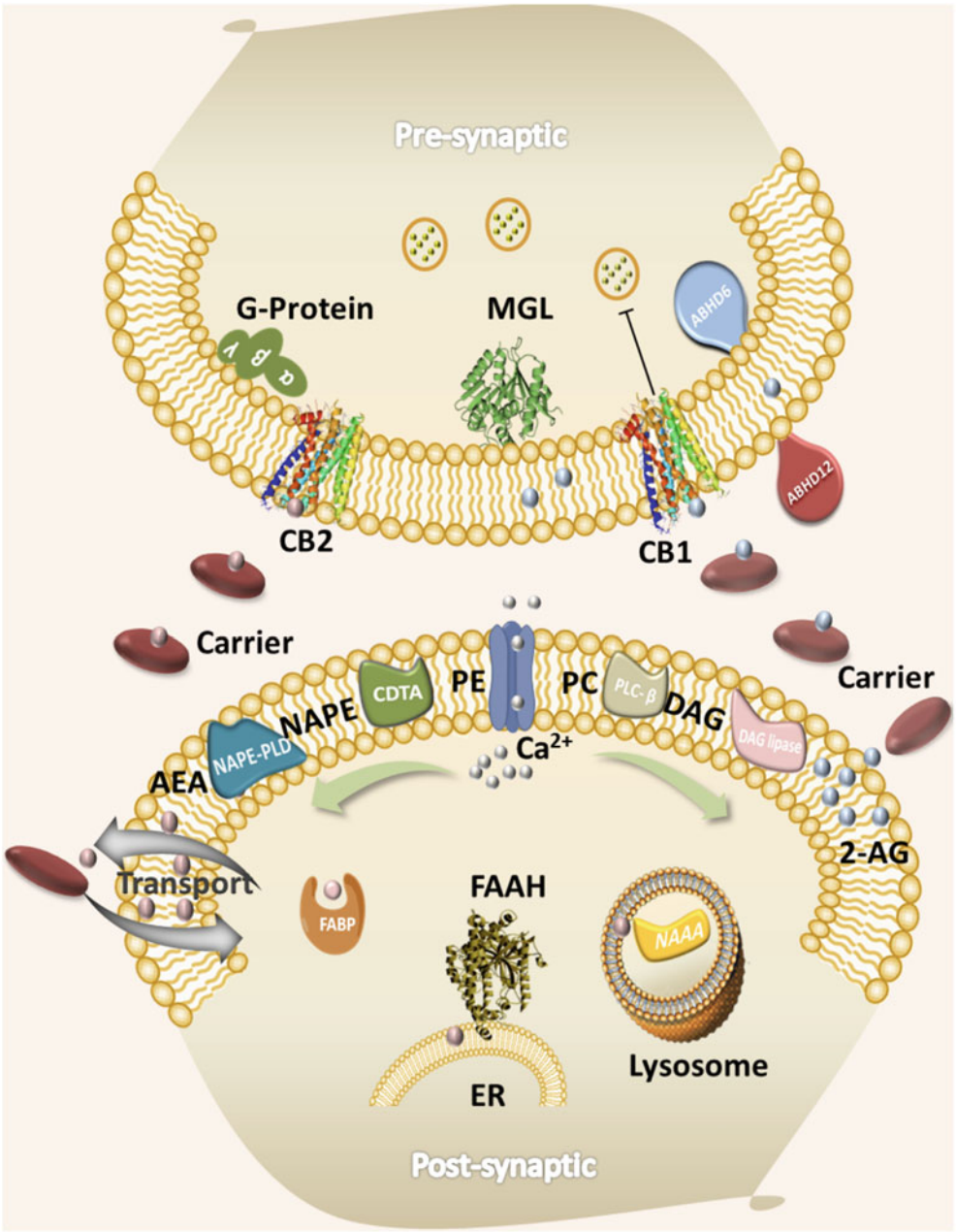

Fig. 3 The feedback loop for the endocannabinoid signaling system is depicted above. *CB1* cannabinoid receptor 1, *CB2* cannabinoid receptor 2, *FAAH* fatty acid amide hydrolase, *MGL* monoacylglycerol lipase, *ABHD6* α-β-hydrolase domain-containing protein 6, *ABHD12* α-β-hydrolase domain-containing protein 12, *NAPE* *N*-arachidonoyl phosphatidylethanolamine, *PE* phosphatidylethanolamine, *PC* phospholipase C, *PD* phospholipase D, *DGL* diacylglycerol lipase, *FABP* fatty acid-binding protein, *AEA* arachidonoyl ethanolamide, *2-AG* 2-arachidonoylglycerol, *ER* endoplasmic reticulum. Source*:* Adapted from Vemuri and Makriyannis (2015)

stimulated in a variety of ways such as neuronal injury or excessive neuronal firing (Basavarajappa 2007).

ECS signaling comes in two forms—tonic and phasic. Tonic signaling establishes eCB tone or a basal level of signaling, while phasic signaling involves temporal perturbations in eCB levels. Researchers have demonstrated that omega-3 fatty acids are essential for the proper regulation of the ECS tone, as these polyunsaturated fatty acids feed directly into the eCB signaling pathways (Lafourcade et al. 2011).

These new discoveries opened the door for characterizing the two cannabinoid receptors, the proteins that modulate their function and the eCB family of compounds that encompass the ECS system. The ECS plays a major role in the regulation of many aspects of human physiology. Today we know that the CB_1 cannabinoid receptor is the most abundant

GPCR in the brain but is also present in many other organs such as the heart, blood vessels, liver, lungs, and the digestive system, as well as fat and sperm cells (Mackie 2008).

4.1 The ECS Explained

The CB_1 receptor belongs to the Class A rhodopsin-like family of GPCRs. It is primarily found in the central nervous system being enriched in the cortex, hippocampus, outflow of the basal ganglia, and cerebellum. There can be both intra- and interspecies differences in the anatomical location of cannabinoid receptors in the ECS. It's important to note that CB_1 in humans is not prevalent in the brain stem or medulla oblongata, the organs responsible for controlling vital autonomic functions such as breathing and heartbeat. This is a strong contributing factor to the safety profile of cannabinoids in humans and the main reason that it is nearly impossible to overdose on THC. However, for dogs this is not true. This receptor is also found to a lesser extent in the periphery of cardiovascular, immune, gastrointestinal, and reproductive tissues. CB_2 receptors are located primarily in immune cells, among them leukocytes and those of the spleen and tonsils (Pertwee 2001). The CB_1 and CB_2 receptors share a significant degree of homology despite being located primarily in the CNS and immune system, respectively. One of the functions of cannabinoid receptors in the immune system is modulation of cytokine release. Activation of B- and T-cell CB_2 receptors by cannabinoids leads to inhibition of adenylyl cyclase in these cells and to a reduced response to immune challenge (Condie et al. 1996). Both CB_1 and CB_2 are coupled to Gi/o proteins and cause a decrease in adenylyl cyclase activity and the cAMP pathway. They also stimulate mitogen-activated protein kinase (MAPK) cascades, modulate ion channels, and modify intracellular calcium levels and hence neurotransmitter release (Howlett and Fleming 1984; Howlett 2002; Marcu and Schechter 2016; Pertwee 2005). Inwardly rectifying potassium channels can also serve as a signaling mechanism for the CB_2 receptor (Griffin et al. 1999; Ho et al. 1999).

Cannabinoid action is not limited to signaling outside of the cell. Fatty acid-binding proteins (FABP) have been demonstrated to be essential for the transport of cannabinoids into the cell where they can interact with cannabinoid receptors on the mitochondrial membrane or they recruit different transcription factors and are translocated into the nucleus where they modify gene expression (Elmes et al. 2015). Additionally there are other proteins that modify cannabinoid signaling such as CRIP1a which inhibits constitutive eCB signaling (Smith et al. 2015).

The cannabinoid receptor CB_1 has also been found intracellularly where it localizes to the mitochondrial membrane and regulates neuronal energy metabolism (Bénard et al. 2012). Mitochondrial CB_1 modifies cellular respiration through its inhibitory actions on soluble adenylyl cyclase and reduced complex I activity in the electron transport chain. Additionally, these mitochondrial receptors may also play a role in the pro-apoptotic mechanisms of cannabinoids in cancer cells.

4.2 Non-cannabinoid Receptor Interactions and Dimerization

Cannabinoids do not solely target CB_1 and CB_2 and can act through other receptor systems. Knockout mice for both of these cannabinoid receptors have been created; however, these mice still exhibit behavioral, biochemical, and electrophysiological responses when cannabinoids are applied, suggesting the presence of other cannabinoid receptor subtypes. For example, recently cannabidiol (CBD) has been shown to act as an inverse agonist at GPR3 and GPR6 (Laun and Song 2017). There are currently a few other GPCRs that are under investigation as potential cannabinoid receptors. One such receptor is GPR18 which has been shown to be activated by the eCB ligand *N*-arachidonoylglycine (NAGly). This receptor is thought to play a key role in microglial activation and hence response to neuronal injury (McHugh et al. 2010).

GPR55 is another putative cannabinoid receptor activated by lysophosphatidylinositol (LPI) and antagonized by CBD that has been shown to play a role in the regulation of bone physiology by regulating osteoclast number and function (Whyte et al. 2009). Similarly to GPR18 it also appears to play a role in the microglial function specifically mediating some of their neuroprotective activities (Kallendrusch et al. 2013). GPR119 is thought to be another component of the ECS. Specifically, this receptor regulates various physiological processes that improve glucose homeostasis, including glucose-dependent insulin secretion from pancreatic β-cells, gastrointestinal incretin hormone secretion, appetite control, epithelial electrolyte homeostasis, gastric emptying, and β-cell proliferation and cytoprotection. These properties make this receptor an attractive candidate as a drug target for metabolic conditions such as diabetes (Mo et al. 2014).

Other potential biological targets interacting with cannabinoids include PPARγ where CBD has been shown to attenuate a-beta inflammation and enhance hippocampal neurogenesis through actions at this site (Esposito et al. 2006). The pain receptor TRPV1 famous for its interactions with the capsaicin found in chili peppers also interacts with cannabinoids to reduce nociception. This target also appears to be implicated in cannabinoid hyperemesis syndrome (CHS) which has recently gained media attention. Treatment of CHS patients with topical capsaicin, a known TRPV1 agonist as well as alternating hot and cold showers, tends to resolve symptoms (Moon et al. 2018).

Opiate-Cannabinoid Receptor Dimerization

CB_1 has been shown to dimerize with both mu and delta opioid receptors (Fig. 4). The mu opioid receptor heterodimer has interesting properties, and when each individual component is activated, eCB signaling is enhanced. However, when both are activated simultaneously, this results in a decrease in signaling. In the case of delta opioid heterodimers, these receptors tend to antagonize each other if one is missing. For example, if the delta opioid receptor is missing from this complex CB_1, signaling increases and vice versa (Fujita et al. 2014).

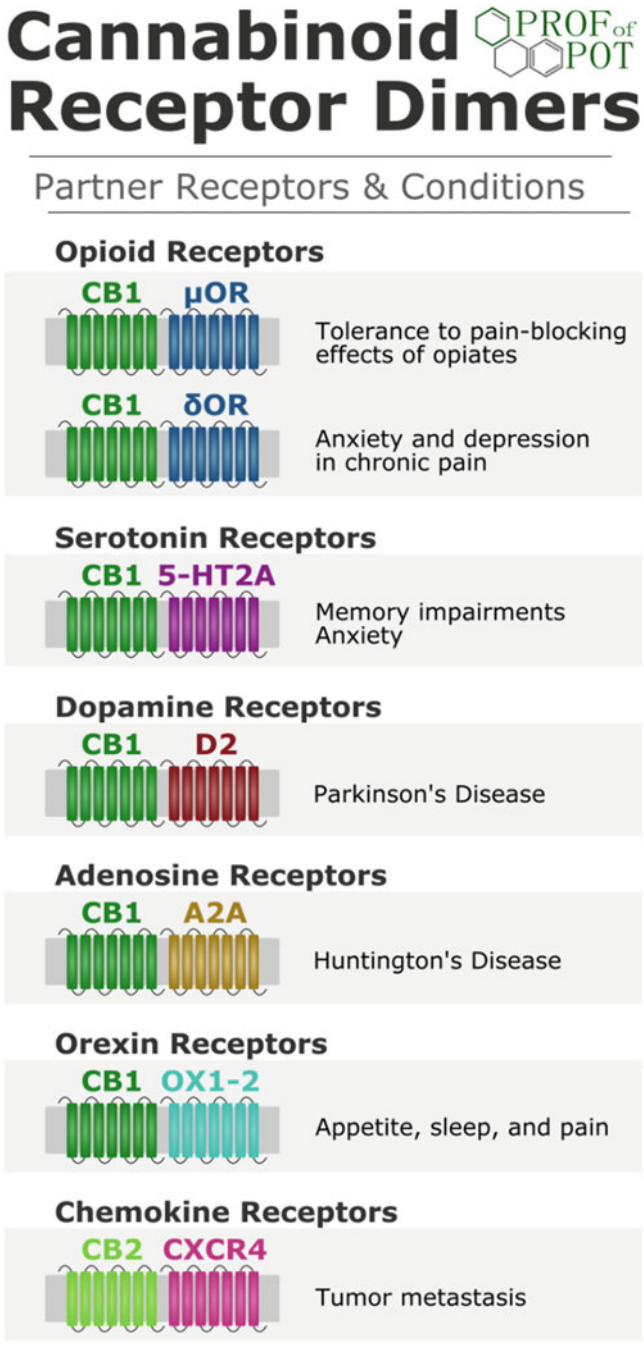

Fig. 4 Cannabinoid receptors have been shown to form dimers with other types of opioid, serotonin, dopamine, adenosine, orexin, and chemokine receptors. With permission from Professor of Pot

Serotonergic System and Cannabinoid Receptor Dimerization

CBD has been shown to produce agonist activity at the serotonin receptor 5-HT1A which may in part account for its anxiolytic properties. The cannabinoid receptor CB_1 has been shown to form heteromeric complexes with the serotonin receptor (5-HT2A). The activity of this receptor complex has been implicated in the memory augmenting properties of cannabinoids (Viñals et al. 2015). Interestingly the prevalence of this specific class of heteromeric complex increases in heavy users as they age and are implicated in certain conditions such as schizophrenia (Galindo et al. 2018). Additionally, cannabinoid agonists also inhibit signaling at the 5-HT3 receptor where they produce antiemetic effects (Fan 1995).

Other Receptor Systems

For an excellent review on this topic, see Prof of Pot's article on "Cannabinoid Receptor Dimerization" from which this figure was taken. See http://profofpot.com/cannabinoid-receptor-dimerization/

4.3 ECS in Health and Disease

Most of what we know about the medical and health benefits of cannabis relates to humans and not animals. Later in the chapter, we will focus on what we do know about cannabis as it relates to animals, but many of the biological interactions occur across the animal species. Below, we highlight many areas that cannabis can be helpful and acts as a starting point to evaluate cannabis as a therapeutic agent in veterinary species.

4.3.1 Sleep

Early studies from the 1970s indicate that THC causes a drastic increase in the production of melatonin, up to 4000% in some instances (Lissoni et al. 1986). It should be no surprise then that it has a profound effect on sleep (Babson et al. 2017). Additionally, cannabinoids affect the different stages of sleep and in particular act as a suppressor of REM sleep (Roehrs and Roth 2017).

4.3.2 Anxiety and Stress

Deficiencies in eCB signaling have been implicated in the etiology of a variety of conditions including PTSD, migraine, and fibromyalgia. In particular circulating levels of eCBs are markedly decreased in all of these disorders. This decline in circulating eCBs also tends to be correlated with anxiety-like

behaviors. A wide variety of studies have demonstrated that chronic environmental stress reliably leads to a down-regulation of CB_1 receptors and reduced levels of AEA increasing levels of 2-AG (Morena et al. 2016).

When tested in animal models, CBD was shown to have anxiolytic properties (Zuardi and Karniol 1983), as confirmed in a number of studies in humans. In an early study using healthy human volunteers subjected to a stressful public speaking test (SPST), a 300 mg dose reduced the volunteers' subjective anxiety to a level comparable to the standard anxiolytic diazepam (Zuardi et al. 1993). Neuroimaging confirmed these effects in follow-up studies (Bergamaschi et al. 2011; Crippa et al. 2009). CBD has also been shown to ameliorate some of the undesirable effects of THC and may be responsible for the improved properties of smoked cannabis compared to THC administration.

4.3.3 Obesity and Metabolic Diseases

Hypothalamic pro-opiomelanocortin neurons are responsible for feelings of satiety. CB_1 receptor activation on these neurons induces activity that causes the effects of these neurons to be silenced. This reduction in satiety can be attributed to the inhibitory effects of cannabinoids on the release of α-melanocyte-stimulating hormone (α-MSH) which normally produces appetite-suppressing effects. Recent studies have also demonstrated an inverse correlation between levels of orexin-A and α-MSH, which led to the discovery that orexin-A induces hyperphagia by increasing levels of the eCB 2-AG. Blockade of the OX-A receptor type 1 mitigated the impairment of α-MSH signaling which may serve as a target for controlling hunger responses (Morello et al. 2016). Because both GABAergic and glutamatergic presynaptic terminals on POMC neurons express CB_1, it is reasonable to assume that the bimodal effect of cannabinoids on feeding is due to the differential sensitivity of GABAergic versus glutamatergic axons to CB_1 activation (Koch et al. 2015).

Cannabis users who are prone to the munchies are generally thought to eat in greater quantities than their cannabis-naive counterparts. On average, users of cannabis display a slimmer waistline and lower BMI than nonusers. While seemingly paradoxical, Le Foll et al. (2013) have found that the prevalence of obesity is lower in regular cannabis users compared to nonusers, even after adjusting for important variables such as age, sex, and tobacco smoking status. A study in 3 T3-L1 adipocytes gives some insight into one possible mechanism by which cannabis users keep their weight in check and shows that treatment of these cells with CBD causes changes in the expression of certain signaling cues that lead to the browning of these cells (Parray and Yun 2016). In addition, cannabis users also display lower levels of fasting insulin and display better insulin sensitivity than their nonusing counterparts (Muniyappa et al. 2013).

Certain cannabinoids have demonstrated effects that may be useful for obesity treatment and prevention. The use of the cannabinoid THCV has correlated with weight loss, as well as decreased body fat and serum leptin concentrations in obese mice (Riedel et al. 2009; Wargent et al. 2013). Additional support for this idea comes from a recent paper by Silvestri et al., in which THCV and CBD were both shown to reduce accumulated lipid levels in adipocytes and in a model of hepatosteatosis (Silvestri et al. 2015).

4.3.4 Cancer

The ECS plays a key role in modulating cell differentiation, cell proliferation, and cell death. Additionally, cannabinoids such as THC stimulate appetite and reduce the emetic responses seen in chemotherapy. These qualities make the ECS an attractive target for use in cancer therapy. Cannabinoids can also be beneficial in certain cancers through their modulation of gene expression. For example, in lung cancers the application of CBD results in an upregulation of the expression of the intracellular adhesion molecules (ICAM) which in turn prevents the metastasis of cancerous cells beyond the tumor site (Haustein et al. 2014). In gliomas, the administration of CBD results in a dose-dependent reduction in the expression of pro-angiogenic factors (Vaccani et al. 2005). THC, when administered in conjunction with CBD, provides synergistic effects in the inhibition of human glioblastoma cell proliferation and survival. Another example of such changes in gene expression occurs in breast cancer cells where McAllister et al. demonstrated that the application of this cannabinoid downregulates the expression of ID-1, which is a large contributor to breast cancer metastasis (McAllister et al. 2007).

4.3.5 Inflammatory Conditions

Inflammation is a highly prevalent condition that contributes to the development and progression of many diseases and health conditions. The ECS has been shown both in vivo and in vitro to be involved in regulating the immune system through its immunomodulatory properties. Cannabinoids have been tested in several experimental models of autoimmune disorders such as multiple sclerosis, rheumatoid arthritis, colitis, and hepatitis.

As the cannabinoid receptor CB_2 is primarily found on the surface of immune cells, it should come as no surprise that these receptors modify the inflammatory response. These receptors have been shown to protect the host from the pathogenesis of these conditions through induction of multiple anti-inflammatory pathways such as suppression of T-cell-mediated immune responses by primarily inducing apoptosis and suppressing inflammatory cytokines and chemokines. However, some studies have shown both pro-inflammatory and anti-inflammatory effects, suggesting different thresholds for different cell populations.

Compounds found in cannabis that reduce inflammation are abundant and diverse. The most abundant phytocannabinoids in cannabis, THC and CBD, both have strong anti-inflammatory properties, while CBC, CBG, and THCV have also demonstrated anti-inflammatory properties. Cannabinoids act as anti-inflammatory agents by inducing apoptosis, inhibiting of cell proliferation, suppressing cytokine production, and inducing T regulatory cells. Apoptotic mechanisms induced by cannabinoids in immune cells include activation of CD95 to induce Bcl-2 and caspase cascades in immune cells. Cannabinoids have also been demonstrated to promote the production of anti-inflammatory interleukins such as IL-10 while inhibiting the production of pro-inflammatory cytokines such as TNF-α in a CB_1-dependent fashion (Klein et al. 2000).

Veterinarians and physicians alike might find it intriguing that nonsteroidal anti-inflammatory drugs (NSAIDs) produce their anti-inflammatory response through interactions with the ECS. Specifically, in the case of acetaminophen, its metabolism by the liver leads to the production of *N*-arachidonoylphenolamine (AM-404) which acts as both a cannabinoid receptor agonist and eCB reuptake inhibitor (Ottani et al. 2006). These interactions also block the conversion of arachidonic acid into inflammation- and pain-promoting prostaglandins (Saliba et al. 2017).

Additionally, certain terpenes also display anti-inflammatory activity. Among the terpenes, α-pinene, β-myrcene, and β-caryophyllene appear to act through prostaglandin receptors (PGE_1 and/or PGE_2) to have an anti-inflammatory effect. Additionally, beta caryophyllene is the only terpene currently known to bind to cannabinoid receptors attenuating inflammation in a CB_2 receptor-dependent fashion (Klauke et al. 2014). Hydrogenated terpenes and cannabinoids often display different characteristics as well and can exist in cannabinoid extracts at low levels as a result of the variety of distillation and refinement techniques being employed (USP #20160324909) (Scialdone 2017)

4.3.6 Pulmonary Effects

Studies performed in the 1970s at the University of California,—Los Angeles, by Donald Tashkin have shown that both inhaled and orally ingested THC produce bronchodilation for up to 2 hours after administration (Tashkin et al. 1974). Further investigations by the Respiratory Pharmacology Laboratory in Paris have shown that CB_1 receptor activation inhibits cholinergic contraction in a concentration-dependent fashion, offering a possible mechanism for the inhibiting of bronchospasms. Additional studies performed at the University of Sao Paulo using CBD have also shown some potential for improving the symptoms of COPD. They found decreased pulmonary inflammation and improvements in lung function in murine models of inflammatory lung disease using the inflammatory agent lipopolysaccharide (Ribeiro et al. 2015). Other findings using murine models have shown that intraperitoneal administration of THC results in a reduction of allergen-induced mucus production (Reddy et al. 2012).

4.3.7 Cardiovascular Effects

Cannabinoids can both increase and decrease blood pressure and heart rate, depending upon the specific context in which they are being used. These effects are only recently becoming better understood as more studies are being conducted regarding these effects (Dewey 1986; Wagner et al. 1998; Niederhoffer and Szabo 1999, 2000).

CBD specifically has been found to have direct effects on arteries, influencing vaso-relaxation, which is clinically observed as a mild hypotensive effect when CBD is administered. CBD has a protective effect against the vascular damage caused by hyperglycemia, as with type 2 diabetes, diabetic angiopathies, and systemic inflammatory processes. It is the antioxidant and anti-inflammatory effects of CBD that mediate these benefits (Stanley et al. 2013).

CBD has been found to have anti-arrhythmic effects in an in vivo rat model of coronary artery occlusion which may not be mediated through the CB_1 receptors found on myocardial cell membranes, but may have other non-receptor-mediated pathways that allow its control over cardiac rhythm (Hepburn et al. 2011).

It was determined in spontaneously hypertensive rats (SHR) that CB_1 antagonists increased blood pressure and left ventricular contractile performance. Additionally, by preventing the degradation of the endocannabinoid anandamide by fatty acid amide hydrolase (FAAH), as is found with CBD, reductions in blood pressure, cardiac contractility, and vascular resistance were found in normotensive rats. These effects were inhibited by CB_1 antagonists. It was found that CB_1 antagonists lower blood pressure much more in the SHR than in normotensive Wistar-Kyoto rats. Additionally, CB_1 receptors are upregulated in the heart and aortic endothelium in SHR versus the normotensive rat cohort (Bátkai et al. 2004).

4.3.8 Antioxidants and Neuroprotection

The NIH holds a patent on cannabinoids as antioxidants and neuroprotectants (Hampson et al. 2003; USP: 6630507). THC, CBD, and CBG have been shown to have antioxidant properties (Hampson et al. 1998; Borrelli et al. 2013). Through their actions as antioxidants, cannabinoids can neutralize reactive oxygen species. THC and CBD were both found to reduce ROS in vitro, with similar potency to known antioxidants such as ascorbate and butylated hydroxytoluene. Additionally, by inhibiting voltage-gated calcium channels, cannabinoids can inhibit the release of glutamate, hence curbing excitotoxicity due to excessive neuronal firing. During periods of ischemia and other traumatic brain events, the

neurotransmitter glutamate is released. Glutamate itself is toxic in excess and can lead to neuronal cell death in a process known as excitotoxic stress. Compounds with antioxidant properties are often neuroprotective, which is believed to be through the reduction of toxic ROS produced during ischemic metabolism. The neuroprotective effect of these compounds was found to be independent of their CB receptor binding activity. CBD in particular has been shown to protect against cerebral ischemic injury (Hayakawa et al. 2008) and also attenuates Alzheimer's-related neuroinflammation in animal models (Esposito et al. 2007).

Plant antioxidants such as ascorbic acid and tocopherols, polyphenolic compounds, terpenes, and numerous mono- and sesquiterpenes are important for human health. Cannabis terpenes with demonstrated antioxidant properties include β-caryophyllene (Calleja et al. 2013), limonene, and β-myrcene (Ciftci et al. 2011). In a mouse study of cerebral ischemia (Ciftci et al. 2011), β-myrcene protected against oxidative stress and histological damage induced by ischemia-reperfusion and is thus an effective neuroprotectant. Further, the compound is suggested as a good candidate for treatment of ischemic stroke.

The ECS is implicated in the etiology of a variety of neurodegenerative conditions, and cannabinoids have been shown to have neuroprotective qualities and the ability to attenuate neuroinflammation and promote neurogenesis (Jiang et al. 2005; Saito et al. 2012; Van der Stelt et al. 2001). In the case of Alzheimer's disease (AD), cannabinoids have shown promise for their ability to clear the toxic beta amyloid (Aβ) plaques that accumulate in the brain in patients with the disease. Considering patients treated with cannabinoids demonstrate improvement in symptoms, it should come as no surprise that a precursor to this debilitating disease is a loss of the body's natural production of eCBs. CBD has also been shown to reduce the expression of genes implicated in the phosphorylation of the tau protein, the hyperphosphorylation of which leads to the formation of neurofibrillary tangles that further contributes to the progression of the disease (Esposito et al. 2006). Furthermore, cannabinoids have been demonstrated to enhance the clearance of Aβ from the brain as well as prevent the inflammatory cascade that is produced by the accumulation of these misfolded proteins intracellularly (Eubanks et al. 2006).

4.3.9 Cannabis, Memory, and the ECS

Michael Pollan in his book, *The Botany of Desire: A Plant's-Eye View of the World*, addresses the short-term memory issues associated with the use of THC and especially the value of cannabis in treating post-traumatic stress disorder (PTSD) (Pollan 2001). Not all memories need to be retained. Some can be traumatic and toxic when recalled, as with PTSD. Pollan poses a question to the reader: "Do you really want to remember all the faces you saw on the subway this morning?"

The ECS acts as a filter for memory, promoting the retention of useful memories and removal of unnecessary ones. This ability to forget is critical for survival and goes awry in certain conditions such as PTSD. The ability of THC to enhance this process of forgetting is in part due to alterations in long-term depression (LTD) in the CA1-CA3 circuit of the hippocampus which is specifically dependent on astroglial CB_1 rather than neuronal cells—suggesting the cleanup of the synapse is impaired (Han et al. 2012). Newer research demonstrates that memory deficits from cannabis are also associated with increased COX-2 activity in the hippocampus (Chen et al. 2013).

It has also been suggested that chronic cannabis use is associated with structural changes in the hippocampus, such as reduced gray matter and alterations in shape. One particular study showed that long-term cannabis users had a 12% reduction in hippocampal volume on average. However, these findings may be flawed since there was a very small sample size used ($n = 15$) (Demirakca et al. 2011; Solowij et al. 2013; Yücel et al. 2008). If these changes are valid, they could have an influence on memory formation in cannabis users. However newer research refutes these findings, showing that daily cannabis use does not cause alterations in adolescents or adults. This study also used a much greater sample size than previous studies, comparing the brain morphology of 29 adult users and nonusers, as well as 50 adolescent users and nonusers using high-resolution MRI scans (Weiland et al. 2015). Other follow-up studies shed some light on the reasons behind these discrepancies by demonstrating that these changes in hippocampal volume and morphology are ablated when co-administered with CBD, displaying a protective effect of this cannabinoid against THC-induced changes (Yücel et al. 2016). Beale et al. examined this effect further and found that its restorative effects were limited to only certain hippocampal regions, specifically the left subicular complex (parasubiculum, presubiculum, and subiculum) (Beale et al. 2018).

4.3.10 Clinical Endocannabinoid Deficiency (CECD)

Deficiencies in eCB signaling have been implicated in the etiology of a variety of conditions including PTSD, migraine, and fibromyalgia (Russo 2016a, b). There are known mutations in ECS genes that contribute to such deficiencies. For example, patients with mutations in CNR1 and DAGLA exhibit CECD phenotypes (Smith et al. 2017). Another example of this is seen in IBS patients where the variant rs806378 *CT/TT* in the CNR1 gene showed a statistically

significant association with rates of colonic transit (Camilleri et al. 2013). Specifically, in the context of PTSD, fear extinction is impaired in patients who were homozygous for the CNR1 variant rs2180619 (Heitland et al. 2012).

In particular circulating levels of eCBs are markedly decreased in all of these disorders. This decline in circulating eCBs also tends to be correlated with anxiety like behaviors. A wide variety of studies have demonstrated that chronic environmental stress reliably leads to a downregulation of CB_1 receptors and reduced levels of 2-AG and AEA (Hill et al. 2009). Equally as predictable is a transient increase in 2-AG in the amygdala following chronic stress showing increases after 30 minutes that return to baseline after 60 min (Patel et al. 2009).

4.3.11 Limitations of the ECS as a Therapeutic Target

The involvement of the ECS in such a wide variety of physiological processes makes it enticing from a practitioner's perspective to understand how to manipulate this system. While the ECS can be viewed as a panacea for the treatment of various conditions, it is not without limitations. In particular, cannabinoid receptor agonists such as THC carry psychoactive effects which can be undesirable in certain populations. Additionally, persistent activation of the cannabinoid receptors results in receptor desensitization which ultimately leads to tolerance and the need for higher dosages, which also increases the likelihood of adverse responses. Unintended consequences of ECS stimulation include memory deficits and the development of withdrawal symptoms. One drawback of prolonged ECS stimulation that has recently gained attention is the development of cannabinoid hyperemesis syndrome (Chang and Windish 2009). This disorder only develops in certain subsets of the population and results in cyclical vomiting that can only be absolved by the administration of alternating hot and cold showers or a TRPV1 agonist such as capsaicin. One such focus area of research was that of appetite and metabolism and attempts to harness this avenue took form as a drug called SR141716A (Rimonabant), a CB_1 receptor inverse agonist. The use of rimonabant had negative outcomes, causing some to have negative thoughts and ideations of suicide, with some patients actually carrying it out. It was later discovered that such issues were attributed with effects on the central nervous system; therefore researchers are now working on developing CB_1 inverse agonists that are restricted to the periphery. There is also the possibility of synthesis issues producing off-target by-products which is still currently under investigation by the FDA.

4.4 Veterinary ECS: Our Current State of Knowledge

The CB_1 receptor is highly conserved across all mammalian species, but there are significant primary sequence differences that have been discovered between the human and rat cannabinoid CB_2 receptors and the newly cloned canine cannabinoid receptor, CB_2. It was found that the binding affinities for canine CB_2 receptor were 30 times less than those measured for human and rat CB_2 receptors. The functional properties of the cannabinoid CB_2 receptor were found to be highly dependent upon the receptor expression level and the nature of the signaling pathway selected (Ndong et al. 2011).

4.4.1 Anatomical Localization of Cannabinoid Receptors in the Dog

Cannabinoid Receptor 1

A recent study used immunohistochemistry to anatomically localize the CB_1 receptor in the normal canine nervous system. Nervous systems were examined from a healthy 4-week-old puppy, three 6-month-old dogs, and one 10-year-old dog. Strong "dot-like immunoreactivity" was found in the neutrophils of the cerebral cortex, *cornu ammonis* (CA), and dentate gyrus of the hippocampus, midbrain, cerebellum, medulla oblongata, and gray matter of the spinal cord. Dense CB_1 expression was found in fibers of the globus pallidus and substantia nigra surrounding immunonegative neurons. Astrocytes were consistently positive in all examined regions. In the PNS, CB_1 immunohistochemistry stained neurons and satellite cells of the dorsal root ganglia and myelinating Schwann cells in the PNS.

The younger dog examined had a lower general CB_1 expression in the brain, showing that the density of receptor expression was less than observed in human fetal and neonatal brain tissue. Lower CB_1 expression has been found in aged rats in specific regions, most prominent being the cerebellum, cerebral cortex, and basal ganglia and less prominent in the hippocampus. This reduction in CB_1 density with age in these rats was consistent with the findings in the older dog examined in this study (Freundt-Revilla et al. 2017). Previous studies have identified CB_1 receptors in salivary glands (Dall'Aglio et al. 2010), hair follicles (Mercati et al. 2012), skin, and hippocampus in dogs (Campora et al. 2012).

Immunohistochemistry was used to study the localization of CB_1 receptors on developing canine embryo (30 days old) with a commercially available antibody. CB_1 receptor immunoreactivity was found primarily in epithelial tissues and included most structures of the central and peripheral nervous system, inner ear, olfactory epithelium and related structures, eye, and thyroid gland (Pirone et al. 2015).

Canine CB_1 Receptor Localization
- Cytoplasm of basal and suprabasal layer cells.
- Hair follicle inner epithelial root sheaths and arrector pili muscles.
- Undifferentiated sebocytes at the periphery of sebaceous glands.
- Secretory and ductal cells of sweat glands.
- Mast cells and fibroblasts.
- Upregulated in atopic dermatitis.

Cannabinoid Receptor 2

As compared to human skin samples, clinically normal dogs have a homogeneous distribution of CB_1 and CB_2 receptors in all epidermal layers. In human, CB_1 is mainly detected in epidermal spinosum and granulosum layers, and CB_2 is detected mainly in basal keratocytes. Both CB_1 and CB_2 receptors have been found in the skin of healthy dogs and dogs with atopic dermatitis. The epidermis of dogs is thinner than that of humans (2–3 nucleated layers in the dog versus 6–7 in the human), which might account for this difference. In dogs with atopic dermatitis, hyperplastic epidermal changes were found, with strong CB_1 and CB_2 immunoreactivity in suprabasal keratinocytes and weak CB_1 and strong CB_2 immunoreactivity in basal keratinocytes indicating upregulation of these receptors during inflammation. CB_1 and CB_2 agonists decrease mast cell degranulation (Campora et al. 2012). To summarize, cannabinoid receptor localization on the skin of the dog was found in cytoplasm of epidermal and follicular keratinocytes, sweat and sebaceous gland epithelial cells, and the mesenchymal dermal cells

Canine CB_2 Receptor Localization
- Epidermis.
- Cytoplasm of cells in the basal and suprabasal layers.
- Hair follicles in the basal and suprabasal cells of the outer and inner epithelial root sheaths.
- Mild immunoreactivity in cells of arrector pili muscles and secretory and ductal cells of sweat glands.
- Sebaceous glands in the cytoplasm and peripheral reserve cells.
- Mast cells, fibroblasts, and endothelial cells.
- Lymph nodes.
- Strong B-cell zone immunoreactivity mainly in germinal centers of secondary follicles.
- Upregulated in atopic dermatitis.

4.4.2 Invertebrate ECS

The two cannabinoid receptors, CB_1 and CB_2, have been found in mammals, birds, reptiles, and fish. In a study of seven representative species of invertebrates, McPartland used tritiated ligand binding assays to characterize the cannabinoid receptors in *Ciona intestinalis* (Deuterostomia), *Lumbricus terrestris* (Lophotrochozoa), *Peripatoides novaezealandiae* (Onychophora), *Jasus edwardi* (Crustacea), *Panagrellus redivivus* (Nematoda) [the beer mat nematode], *Actinothoe albocincta* [white striped anemone] (Cnidaria), and *Tethya aurantium* (Porifera) [Orange Puffball sponge] (McPartland et al. 2006).

Cannabinoid binding was detected in all species studied except for the sea anemone (*A. albocincta*) and sponge (*T. aurantium*). The receptors were consistent with CB_1 receptors but not CB_2 receptors. Three of the organisms tested, earthworm (*L. terrestris*), velvet worm (*P. novaezealandiae*), and mat nematode (*P. redivivus*), were compared to a standard CB_1 ortholog in rat cerebellar tissue. A high affinity binding interaction was observed at various concentrations characteristic of CB_1 receptors.

The authors of this study hypothesize that cannabinoid receptors evolved in the last common ancestor of bilaterians, with secondary loss in insects and other clades. After conducting a systematic literature review, the authors found that cannabinoid receptors have been identified in sea urchins, leeches, earthworms, hydra, lobster (*H. americanus and J. edwardi*), and the beer mat nematode (*P. redivivus*), but not the nematode (*C. elegans*). No binding was observed in sponges (Porifera).

In a separate study, McPartland found that insects (*Apis mellifera* [western honey bee], *Drosophila melanogaster* [common fruit fly], *Gerris marginatus* [water strider], *Spodoptera frugiperda* [fall armyworm moth larva], and *Zophobas atratus* [darkling beetle]) are devoid of cannabinoid receptors. This loss of CB receptors is unique to comparative neurobiology, in that no other known mammalian neuroreceptor has been found to be missing in insects (Ecdysozoa). The authors suggest that the lack of cannabinoid receptors in insects is due to their lack of ligands, in that insects produce little or no arachidonic acid, the precursor to the biosynthesis of endocannabinoids (McPartland et al. 2001).

5 *Cannabis sativa* Chemistry

The fascinating effects that cannabis plant constituents have on the human experience have led to the discovery of 113 phytocannabinoids, each with a unique pharmacological profile (*C. sativa*. Pertwee *Handbook of Cannabis* 2014; Radwan et al. 2008). There is an emerging interest in another pharmacologically active group of 140 terpenes that have been identified in cannabis.

Terpenes have been shown to synergistically modulate the therapeutic effects of cannabinoids and also define the aromatic scent profile of the differing cultivars (Brenneisen and ElSohly 1988). The strain-specific effects are attributed to the unique chemotaxonomy of cannabinoids and terpenes (Russo 2011). Over 750 naturally produced compounds have been

found in cannabis (Upton et al. 2014), which may be divided into many different chemical classes. Covering each component of *C. sativa* is beyond the scope of this chapter and not relevant to the therapeutic activity of cannabis. An excellent review of *C. sativa* chemistry can be found in the body of work compiled in Table 1 (Brenneisen and ElSohly 1988; Callaway 2004; ElSohly and Slade 2005; Turner et al. 1980; Upton et al. 2014).

5.1 Phytocannabinoids

Plant-derived cannabinoids, or phytocannabinoids, refer to chemicals derived from botanical sources that interact with the ECS. The term is often synonymous with pharmacologically active compounds isolated from *Cannabis sativa*. Phytocannabinoids differ from eCBs with regard to their long-lived pharmacokinetic profile when compared to the relatively fast acting, short, and intermittent lifetime of the eCBs.

Of the 113 cannabinoids identified in *C. sativa*, the majority can be catalogued as analogs of Δ^9-tetrahydrocannabinol (Δ^9-THC), CBD, cannabichromene (CBC), cannabigerol (CBG), cannabinol (CBN), cannabicyclol (CBL), cannabielsoin (CBE), and cannabitriol (CBT). There are common points of variability on the phytocannabinoid scaffold that are highlighted in Fig. 5. The points of structural variability existing across the phytocannabinoid classes follow some general trends and quickly add up to over 100 potential cannabinoids when repeated across the common analogs shown in Fig. 5. The length of the alkyl side chain is a common site of variability and can range from one to five carbons in length (e.g., R^1, CBD, Fig. 5). The most recognizable and abundant cannabinoids contain *n*-pentyl side chains ($n = 5$), but *n*-butyl, *n*-propyl, ethyl, and methyl side chains have also been identified at lower concentrations (ElSohly and Slade 2005; Turner et al. 1980). Cannabivarins contain a 3-carbon side chain and are also commonly observed. The presence or absence of a carboxylic acid functional group is another point of common structural variability. Though THC and CBD are the most well-known cannabinoids, it is important to note that they are not synthesized in the cannabis flower directly. Rather, they are synthesized in their acidic forms in the plant, which are tetrahydrocannabinolic acid (THCA, R = COOH, R^1 = *n*-pentyl, R^2 = H) and cannabidiolic acid (CBDA, R = COOH, R^1 = *n*-pentyl). These compounds can be converted into THC and CBD by heating. The application of increased heat speeds up the reaction in which a CO_2 molecule is released from THCA to form THC in a process termed decarboxylation. Though some people refer to this reaction as "activating" the cannabinoids, it is a bit of a misnomer, as the acidic versions of these compounds also have been shown to induce medical benefits themselves, most notably anti-inflammation properties (Veress et al. 1990). Microbial hydroxylated forms also exist at low levels and remain relatively unstudied (Rashidi et al. 2009). The choice to decarboxylate can have important effects on the therapeutic application.

Table 1 Chemical components identified in *Cannabis sativa*

Chemical class	Identified compounds
Terpenes	140
Cannabinoids	113
Hydrocarbons	50
Sugars and related compounds	34
Nitrogenous compounds	27
Non-cannabinoid phenols	25
Fatty acids	23
Flavonoids	23
Simple acids	20
Simple ketones	13
Simple ester and lactones	13
Simple aldehydes	12
Proteins, enzymes, and glycoproteins	11
Steroids	11
Elements	9
Simple alcohols	7
Vitamins	3
Pigments	2

Tetrahydrocannabinol, or Δ^9-THC, is the main cannabinoid responsible for the characteristic psychoactive effects of cannabis with which most people are familiar. The binding activity of THC to the CB_1 receptor can cause downstream physiological changes such as mental euphoria, increased appetite, and slower cognitive functioning. A synthetic isolated version of THC called Marinol was approved by the FDA in the late 1990s for its antiemetic effects and prescribed to cancer patients undergoing chemotherapy.

Cannabidiol, or CBD, has received a surge of interest in the last decade by many interested parties, ranging from academic researchers to pharmaceutical drug developers to even nutritional supplement manufacturers. Part of this is due to the well-studied medical benefits. At the time of writing of this chapter, a CBD-based pharmaceutical drug (Epidiolex) has just been approved as a drug by the FDA on June 25, 2018.

Starting in the 1970s, investigators identified CBD's anticonvulsant properties in rats (Consroe and Wolkin 1977) and conducted an initial testing in humans in small groups (Carlini and Cunha 1981). This was followed by a larger human study using 900–1200 mg/kg daily which showed that CBD was effective in treating seizure states (Trumbly 1990). Overall, CBD exhibits the most reliable anticonvulsant effects of cannabis constituents. In very recent reports describing its use in children experiencing epileptiform

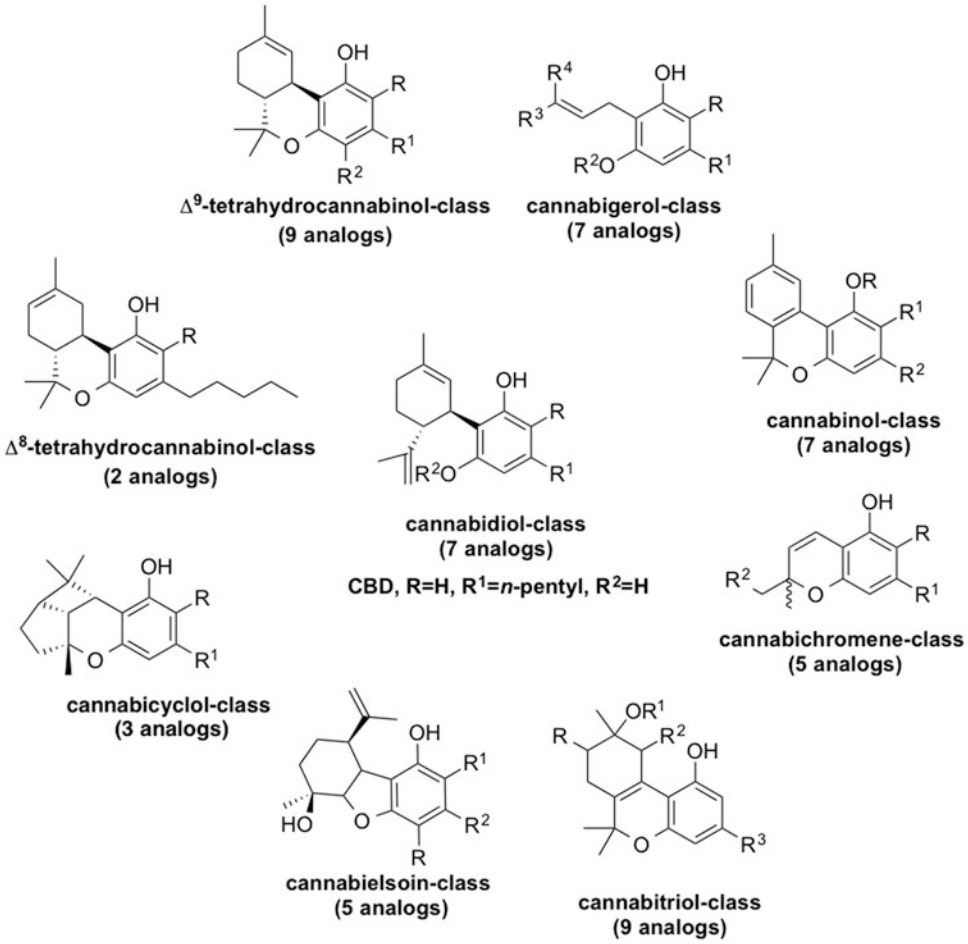

Fig. 5 Common cannabinoid analogs found in *Cannabis sativa*. This figure illustrates the common points of variability within each class and how many analogs have been discovered

conditions, CBD was shown to greatly ameliorate these effects. Also, initial results from a broad clinical evaluation of CBD in a seizure-associated condition in children are very promising. In epileptic patients there is a dysregulation of neuronal firing resulting in seizures and excitotoxicity. Increased levels of AEA have been found in the cerebrospinal fluid of dogs suffering from idiopathic epilepsy as compared to healthy dogs (Gesell et al. 2013).

Ligresti et al. (2006) examined the antitumor effects of a variety of cannabinoids, including both neutral and acidic forms. While CBD was determined to be the most potent antitumor cannabinoid tested, CBD-A was the least. The activity of CBD was due to its capability of inducing apoptosis via direct or indirect activation of CB_2 and potential vanilloid type 1 receptors (Ligresti et al. 2006) (Fig. 6).

A key structural distinction between THC and CBD is the freely rotatable bridge between the aromatic and nonaromatic cyclic systems that only exists in CBD. THC and CBD both have the same empirical formula as well as molecular weight. However, THC is conformationally constrained in a way that doesn't allow this region of the molecule to "flop" around. They are different in three-dimensional space, but CBD also has an additional free hydroxyl group that results in distinct

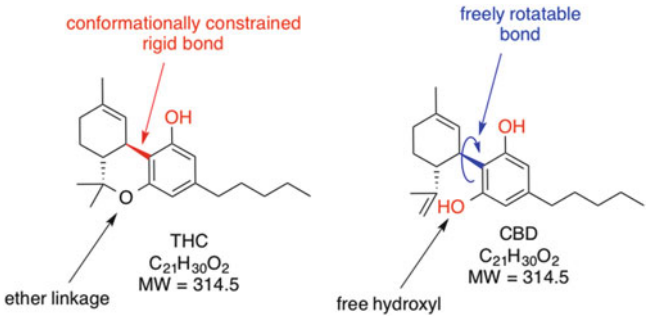

Fig. 6 Illustrates that CBD and THC have the same empirical formula and molecular weight. However, THC is conformationally constrained through an ether linkage and interacts with the receptors in a slightly different way compared to CBD

interactions with CB receptors and other biologically active targets.

(−) Δ^9-Tetrahydrocannabivarin (THCV) is the propyl analog of THC in which the 5-carbon side chain is shortened by two methylene units. Its pharmacological properties have been studied since the early 1970s when it was recognized that it behaves as a significantly weaker agonist (approximate fivefold) compared to THC.

Thus, as with THC, THCV produces analgesic but also cataleptic effects in animals (Hill et al. 2012; Pertwee 2008). These effects can be antagonized by synthetic CB_1 antagonists such as SR1716A or AM251. This property of being a weaker agonist than THC is consistent with extensive medicinal chemistry work showing the decreased potency of the cannabivarin family relative to the longer side chain analogs. Maximum potency is observed with side chains of 7–8 carbons (Makriyannis 2014).

Although THCV can behave as a CB_1 agonist in vitro, it can also act as a CB_1 antagonist capable of blocking the effects of more potent synthetic CB_1 agonists as well as THC and the eCBs AEA and 2-AG. For this reason, Δ^9-THCV can act as an in vivo CB_1 antagonist. At the CB_2 receptor, Δ^9-THCV behaves as a partial agonist and is capable of activating this receptor, although only to a limited extent. CB_2 receptor activation has been shown to be associated with attenuation of inflammation. The antiepileptic effects of this compound are being tested in humans currently.

Cannabigerol (CBG), in its acid form (CBGA), is the principal precursor and convergent intermediate of all phytocannabinoids. CBG is non-psychoactive and is a relatively weak partial agonist for both CB_1 and CB_2. Because of its low cannabinoid receptor potency, it can functionally antagonize the CB_1 effects of THC. It has been shown to relieve intraocular pressure, potentially useful in the treatment of glaucoma. Additionally, its antioxidant and anti-inflammatory properties make it a potential candidate for inflammatory bowel disease. Recent evidence identifies CBG as a potential candidate for the treatment of colon cancer (Ligresti et al. 2006).

There is some information on *tetrahydrocannabinolic acid (Δ^9-THCA)* and *cannabidiolic acid (CBDA)*, the two naturally non-psychotropic precursors of Δ^9-THC and CBD, respectively, that shows that their therapeutic value is derived from mechanisms other than classical CB_1/CB_2 receptor binding (Ahmed et al. 2008). THCA in vitro is capable of modulating the functions of two TRP channel receptors acting as a potent TRPA1 agonist and TRPM8 antagonist, inhibiting both cyclooxygenase enzymes, COX-1 and COX-2. When tested in rat models of nausea, THCA appears to be a better alternative for treating nausea and vomiting associated with cancer chemotherapy (Rock et al. 2013, 2014). THCA has also been shown to reduce levels of TNF-α in vitro, suggesting a mechanism for immune modulation (Ligresti et al. 2006). This reduction in TNF-α has been demonstrated in culture supernatants from U937 macrophages and peripheral blood macrophages after stimulation with LPS in a dose-dependent manner (Verhoeckx et al. 2006). Additionally, THCA has also been demonstrated to have potent activity as a PPARγ agonist (Nadal et al. 2017). However, these findings may only have implications for the periphery as there is some evidence to suggest that THCA's access to the central nervous system is restricted due to interaction with the BBB (Moreno-Sanz 2016). CBDA was shown to be a selective inhibitor of COX-2 (Takeda et al. 2008), implying anti-inflammatory activity. It also has been demonstrated to be a potent agonist at 5-HT1A (Bolognini et al. 2013). Unlike its congener, CBDA effectively reduced anticipatory nausea and may be useful against acute nausea induced by chemotherapy (Rock and Parker 2013).

Cannabinol (CBN) is a degradation product of Δ^9-THC caused by oxidation; therefore, it isn't surprising that it was the first phytocannabinoid to be isolated in pure form. CBN can activate both CB_1 and CB_2 receptors with a potency approximately ten-fold lower than Δ^9-THC. It can thus be viewed as a weak Δ^9-THC relative (Izzo et al. 2009). Less is known about the pharmacology of the diverse array of low abundance phytocannabinoids found in cannabis. Research on rare phytocannabinoids is rapidly expanding and an active area of investigation (Hartsel et al. 2016).

5.2 Chemotaxonomy of *C. sativa*

Chemical phenotypes (chemotypes) can be useful to classify *C. sativa* as drug- or fiber-type varieties. The United Nations Office on Drugs and Crime categorizes *C. sativa* into three chemotypes based on the proportion of THC and CBN relative to CBD [Eq. (1)] (Drugs 2009). Chemotype I (drug-type) cultivars are characterized by X values greater than one, while values less than one are representative of chemotype III cultivars (fiber-type). CBD-rich cultivars containing low levels of THC are regarded as fiber-type, or chemotype III cultivars. The evidence suggests that drug-type or chemotype I *C. sativa* cultivars with high levels of Δ^9-THC originated from below the 30°N latitude (Hillig and Mahlberg 2004). Equivalent levels of Δ^9-THC and CBD are known as chemotype III and are typically found above the 30 °N latitude. Hillig and Mahlberg (2004) used a statistical approach to define chemotaxonomic trends in *C. sativa* and found that most cultivars fell outside of the arbitrary values set by the United Nations Office on Drugs and Crime. They demonstrated that most cultivars clustered into either chemotype I ($X > 10$), chemotype II ($0.2 < X < 10$), or chemotype III ($X < 0.2$). The relative cannabinoid levels in *C. sativa* remain constant from the seedling stage throughout the plant life cycle (Broséus et al. 2010), making it possible to determine the chemotype at early development stages prior to flowering (Barni-Comparini et al. 1984; Vogelmann et al.

Equation 1 Chemotaxonomy is calculated by dividing the sum of THC and CBN concentration by the CBD concentration

$$X = \frac{[\mathrm{THC}] + [\mathrm{CBN}]}{[\mathrm{CBD}]}$$

1988). To date, 52 hemp cultivars that fall below the 0.3% legal limit for hemp have been approved for commercial use by the European Union (Directive 2013). These registered varieties originate from high latitude European nations, which are not acclimated for equatorial regions of the world. Little is known about the viability of the European cultivars in the United States, and this is currently being examined by agronomists. Several high CBD strains have come online in the United States in recent years that are being legally cultivated under the Farm Bill. Some of these strains have CBD contents above 15% and render traditional European cultivars obsolete for oil and resin production.

Over recent decades the cannabis gene pool has undergone selective pressure to maximize THC production at the expense of CBD and other minor or non-psychoactive cannabinoids. The observation that different high THC cannabis cultivars produce a variety of effects prompted researchers to look further into the other chemical constituents that may be impacting the therapeutic effects. It turns out that there is a wide spectrum of effects encountered when different terpene profiles are administered with THC. This has been a major focus of research as of late, and there are numerous publications that demonstrate the variability and unique signatures of terpene profiles in differing cultivars (Fischedick et al. 2010; Henry 2017; Lewis et al. 2018).

5.3 Cannabimimetics: Cannabinoids from non-*C. sativa* Species

Aside from cannabis, there are a variety of other plants that produce compounds that interact with the body's ECS. These compounds are classified as cannabimimetics or cannabinoid-mimicking compounds. Some of the plants that produce cannabimimetics are widely used in herbal medicine, and some are even part of our regular diet.

There is a class of lipid compounds that is widespread in the plant kingdom called *N*-acylethanolamines (NAE). Rather than directly binding to the body's cannabinoid receptors, these compounds inhibit the actions of the eCB-degrading enzymes such as the fatty acid amide hydrolase (FAAH) (Gertsch 2017). There is also a triterpenoid compound called pristimerin found in a family of shrubs called Celastraceae that reversibly inhibits another eCB-degrading enzyme, monoacylglycerol lipase (MAGL) (King et al. 2009). These compounds are significant because this type of activity indicates that their presence can modify eCB tone. Newer research on compounds with similar structural components suggests that *N*-alkylamides and their derivatives could also interact with the putative third cannabinoid receptor GPR55 (Dalle Carbonare et al. 2008).

Studies on *black truffles* have shown that eCBs are not just unique to mammals. The enzymes required for producing anandamide as well as the compound itself are found in certain species of truffles (Pacioni et al. 2015).

Researchers out of Zurich have been investigating a class of compounds in *Echinacea* called *N*-alkylamides, which possess immunomodulatory properties (Gertsch et al. 2008b; Raduner et al. 2006). In particular, *Echinacea* roots produce endocannabinoid-like molecules that have been shown to bind to cannabinoid receptors in rodents (Woelkart and Bauer 2007). Specifically, *N*-isobutylamide has been shown to bind to both cannabinoid receptors but shows preference for CB_2. Its actions there have been shown to cause a decrease in the expression of the pro-inflammatory mediator TNF-α (Gertsch et al. 2004).

South African *Helichrysum umbraculigerum* has been shown to contain CBGA, CBG, and other prenylated dibenzyls similar to cannabinoids in *C. sativa*. Cannabinoid-like molecules derived from *Helichrysum* and other Asteracea genera generally have a phenethyl group at the *n*-pentyl position and have been used in traditional medicine to treat a host of inflammation and infections (Pollastro et al. 2017).

Piper methysticum also known as *kava kava* contains yangonin, a kavalactone that displays significant binding affinity for CB_1 (Russo 2016a, b).

Liverwort (*Radula marginata*) has also been shown to produce a compound called perrottetinenic acid, which closely resembles Δ^9-THC (Toyota et al. 2002).

The Chinese *Rhododendron has* also been shown to produce compounds that are structurally similar to the cannabinoids CBC, CBL, and cannabicitran, although their pharmacological activities at cannabinoid receptors have yet to be explored (Iwata and Kitanaka 2011).

5.4 Cannabis Terpenes

Terpenes are the aromatic compounds in cannabis that contribute to its unique scents and aromas. Over 100 different mono-, di-, and sesquiterpene compounds are produced within the resinous trichomes of cannabis flowers. Terpenes are produced alongside cannabinoids, which share common biosynthetic pathways and intermediates (Fig. 7).

Terpenes are thought to modulate the therapeutic effects of the cannabinoids and are termed the ensemble effect, a reference in literature to a large body of evidence suggesting that whole plant cannabis is more effective than isolated cannabinoid compounds by themselves. The complex aromatic profile of terpenes is often a distinguishing factor between cannabis strains and contribute the pluripotent physiological effects. Despite the fact that the terpene profile can

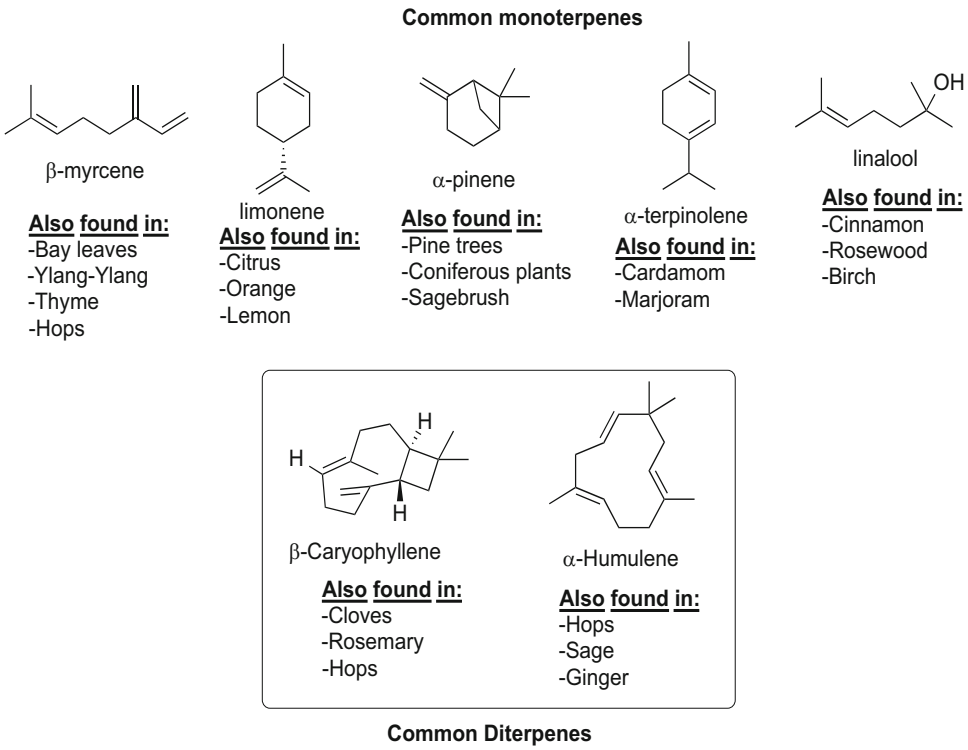

Fig. 7 Showcases five common monoterpenes and two diterpenes found in *Cannabis sativa*

make a cannabis strain unique, terpenes themselves are commonly found in other plants. In fact, many of these terpenes have been used in aromatherapy for millennia. In this section, we will cover five of the most common monoterpenes and two common diterpenes.

β-Caryophyllene is the primary sesquiterpene in black pepper that contributes to its spicy flavor. It is also a major constituent of cloves, hops, rosemary, and, of course, cannabis. Certain strains of cannabis such as Girl Scout Cookies can have levels of β-caryophyllene as high as 5% by mass. It is unique in that it has been shown to directly interact and bind with the CB_2 receptor, making it one of the most ubiquitous non-cannabis cannabinoids found in nature. CB_2, particularly in peripheral tissues in the body, is a therapeutic target for treatment of inflammation, pain, atherosclerosis, and osteoporosis (Gertsch et al. 2008a, b). β-Caryophyllene itself has shown promising results in animal models for colitis, osteoarthritis, diabetes, anxiety, depression, liver fibrosis, and cerebral ischemia. In support of the entourage effect hypothesis, co-administration of β-caryophyllene with the chemotherapy drug Paclitaxel on in vitro cancer cells stimulated increased cancer cell death and decreased tumor growth. β-Caryophyllene has received GRAS status by the FDA, meaning it is generally recognized as safe for oral consumption in humans.

Myrcene is known as the active sedating principle of hops, lemon grass, basil, and mangoes. *Myrcia sphaerocarpa*, from which myrcene is derived, is a medicinal shrub from Brazil traditionally used to treat diabetes, diarrhea, dysentery, and hypertension (Ulbricht 2011). Myrcene's earthy, fruity, and clove-like aroma is pungent in higher concentrations and commonly used in culinary and perfume preparations. Myrcene has been shown to enhance transdermal absorption (Schmitt et al. 2009). It also has a significant analgesic effect, which is blocked by the action of naloxone, an opioid antagonist, suggesting a mechanism of action through the opioid receptor (Rao et al. 1990). Myrcene lacks affinity for opioid receptors pointing to alpha 2-adrenoceptor-stimulated release of endogenous opiates. No tolerance was observed after repeated dosing in rats, which is in contrast to morphine (Lorenzetti et al. 1991). Myrcene was a sedative comparable to phenobarbital at very high doses in rats (Do Vale et al. 2002), an effect increased by simultaneous administration of citral, a mixture of other terpenes. Myrcene was also shown to improve glucose tolerance in alloxan diabetic rats comparable to metformin (Al-Omari 2007), without an effect on glucose levels in normal rats. Further, myrcene showed powerful anti-inflammatory and anti-catabolic effects in a human chondrocyte model of osteoarthritis (Rufino et al. 2015). Myrcene is the subject of a broad array of current research given that inflammation is the underlying cause of numerous diseases.

Limonene is one of the most abundant terpenes in cannabis and has been reported at concentrations up to 16% of the essential oil fraction. Limonene is a monoterpene commonly found in citrus rind and used in perfumes, household cleaners, food, and medicines. Limonene has numerous potential medicinal benefits demonstrated in human and

animal studies. Limonene's antioxidant and anticancer properties make it an excellent dietary source for cancer prevention (Aggarwal and Shishodia 2006). Multiple modes of anticancer activity and chemoprevention were observed for limonene (Crowell and Gould 1994). Perillyl alcohol is a metabolic product or limonene, which is also a subject of numerous cancer-related studies (Prates Ong et al. 2012). Anti-inflammatory effects in models of osteoarthritis (Rufino et al. 2015) and asthma have also been observed (Hirota et al. 2012). Anxiolytic effects in a mouse maze model were not antagonized by flumazenil and comparable to diazepam, implying a non-benzodiazepine biological target (Lima et al. 2013). Earlier results which also demonstrated antidepressant activity via the 5-HT_{1A} receptor pathway (Komiya et al. 2006) are in contrast to anxiolytic effects in the mouse maze model.

Humulene is the characteristic terpene of hops, *Humulus lupulus*, that is also abundant in cannabis. It is also found in sage and ginseng among other plant species. Humulene, also known as α-caryophyllene, is a ring-opened isomer of β-caryophyllene, which lacks in the CB_2 activity of the latter. β-Caryophyllene and humulene have been shown to interact in a synergistic manner in one study (Legault and Pichette 2007). Humulene also possesses powerful anti-inflammatory activity, equal to dexamethasone in an animal model (Fernandes et al. 2007). Both topical and systemic anti-inflammatory properties (Chaves et al. 2008) as well as topical, oral, and aerosol delivery methods as an analgesic have also been observed for humulene (Rogerio et al. 2009). It also has the potential to aid in wound healing (Satsu et al. 2004) from the increase secretion of IL-8, a chemokine with various functions, including promoting angiogenesis.

α-Pinene is a terpene commonly found in pine needles, and its pharmacological properties have been well studied. In particular, α-pinene acts as an acetylcholinesterase (AChE) inhibitor which may aid in memory function (Miyazawa and Yamafuji 2005). It also exhibits anti-inflammatory properties through numerous pathways decreasing levels of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α). It also reduces prostaglandin synthesis through its potent inhibition of prostaglandin E_1 (PGE_1) and like many terpenes also exhibits antimicrobial effects (Nissen et al. 2010; Russo 2011).

Terpinolene carries a woody and floral odor and is commonly found in "sativa" varieties of cannabis. Studies in mice carried out using *Microtoena patchoulii* which has terpinolene as a primary active constituent show that it exhibits sedating properties (Ito and Ito 2011). It also exhibits anticancer properties in the brain cells of rats (Aydin et al. 2013).

Linalool is a terpene commonly found in lavender that produces antianxiety and antidepressant effects. Studies performed in mice suggest that these properties are primarily due to its action on monoaminergic pathways in particular the serotonin receptor 5-HT1A (Chioca et al. 2013; Guzmán-Gutiérrez et al. 2015).

5.5 The Entourage/Ensemble Effect in Cannabis: Why It Matters

The idea of an entourage or ensemble effect in eCB signaling was first proposed by Ben-Shabat in 1998. Since then there have been numerous studies that support this theory. Ben-Shabat's initial work demonstrated this phenomenon with typically inactive fatty acids in the presence of eCBs. A few years later in 2001, McPartland and Russo published a paper titled "Cannabis and Cannabis Extracts: Greater Than the Sum of Their Parts" which extended this entourage concept presenting early evidence that terpenes enhanced the effects of the cannabinoids (McPartland et al. 2001). This work was further refined later by Russo in August of 2011 when he published "Taming THC: Potential cannabis synergy and phytocannabinoid terpene entourage effects" in the *British Journal of Pharmacology* (Russo 2011). This paper proposed that the terpenes in cannabis were capable of modulating the effects of cannabinoids and offered many possible mechanisms by which terpenes could induce this action.

Terpenoids are potent modulators of consciousness and have been used for thousands of years in aromatherapy for a number of conditions. Recent work using essential oils derived from cannabis void of any cannabinoids illustrates such effects where results revealed decreased diastolic blood pressure, increased heart rate, and significant increased skin temperature. There were also changes in the amplitude of alpha, delta, and theta brain waves (Gulluni et al. 2018).

More recent work confirms the concept of an ensemble effect. One such study demonstrates that CBD administered in a whole plant extract rather than an isolated form allows for the bell-shaped dose-response curve to be overcome and results in greatly enhanced therapeutic efficacy in the context of TNF-α production (Gallily et al. 2015).

A number of studies have found that although there is definite clinical potency to the isolated phytocannabinoids such as THC or CBD, when they are provided in a whole plant extract containing terpenes, flavonoids, and other cannabinoids, they have a greater effect. Romano et al. (2014) found that a standardized cannabis extract with a high content of CBD was able to inhibit colon carcinogenesis both in vitro and in vivo. It was found that the whole plant extract reduced cell proliferation in cancerous but not in healthy cells. Pure CBD reduced cell proliferation in a CB_1-sensitive antagonist manner only. In vivo, plant-based CBD reduced the carcinogen azoxymethane (AOM)-induced preneoplastic lesions and polyps as well as tumor growth in

the xenograft model of colon cancer. The authors concluded that plant-based CBD in the whole plant context attenuates colon carcinogenesis and inhibits colorectal cancer cell proliferation via CB_1 and CB_2 receptor activation (Romano et al. 2014).

6 Animal Studies and Veterinary Clinical Trials

For legal reasons, although cannabis for medical uses has been applied in an uncontrolled (and possibly illegal) fashion to pets and farm animals for many years, going back as far as the mid-1800s, there has been no basic, fundamental research into the safety, efficacy, or pharmacokinetics of phytocannabinoids, and specifically CBD, in our domestic or wild species of animals. With the federal legalization of hemp in the 2014 Farm Bill, as mentioned previously, there has been a loosening of the restrictions on research in states that have legal hemp cultivation and commercialization.

As a result of this legal loosening of cannabis research, several veterinary academic institutions have completed or nearly completed studies into the safety of high-dose, long-term use (6 weeks) of CBD in the dog, the relative pharmacokinetics of three different avenues of administration (transmucosal, oral, and transdermal), and the efficacy of CBD use for osteoarthritic pain and refractory epilepsy at a specific dosage. The efficacy studies are prospective, double-blind, placebo-controlled, randomized clinical trials. Additionally, several pet owner surveys/studies have been completed. One has been published in a peer-reviewed journal, and the second has been reviewed and is scheduled for publication later in 2018 by the same peer-reviewed journal. These surveys are from Colorado State University's College of Veterinary Medicine. A summary of the study results from the CSU comparative pharmacokinetics study is reported here. The safety and efficacy studies for epilepsy and osteoarthritis will be published later in 2018. The preliminary data from the two studies are reported here based on presentation of this data from conference proceedings.

Cornell University's College of Veterinary Medicine has just released a pharmacokinetics, safety, and efficacy study of CBD used in dogs with osteoarthritis. This document was presented at the American College of Veterinary Sports Medicine and Rehabilitation track at the World Rehabilitation Summit (IAVRPT) in Knoxville, TN, July 31, 2018. This study's results are reported in this chapter as well. Both the Cornell and CSU studies used "macro-dosages," which are substantially higher (4–5 times) than have been reported as effective by veterinarians and pet owners based on anecdotal and observational data.

6.1 Pet Owner Experiences with Hemp Products

Kogan et al. (2015, 2018) from Colorado State University conducted a survey of pet owners who had visited an e-commerce site to purchase animal hemp products. The survey was intended to inform future research of the best veterinary applications for low THC cannabis, otherwise known as "industrial hemp." This study was designed to determine what hemp products pet owners are purchasing, reasons for their purchases, and the perceived value of these products on their pet's health. An anonymous survey was given to pet owners who bought products from a single online hemp company. A total of 632 responses were recorded, and 58.8% of these indicated they currently use a hemp product for their dog. Most dog owners (77.6%) indicated they use the product for an illness or condition diagnosed by a veterinarian, with the most common conditions including seizures, cancer, anxiety, and arthritis.

Of the total responses, fewer participants indicated they currently use the hemp products for their cat (11.93%), with 81.8% indicating they use the product for an illness or condition diagnosed by a veterinarian. The most common conditions being addressed by the hemp products included cancer, anxiety, and arthritis. This study provides a guide to researchers seeking to perform clinical studies on hemp in terms of its best areas of efficacy and potential adverse outcomes with its use. The authors suggest, for this first evidence-based publication in the veterinary realm of the clinical effects of cannabinoids, to consider controlled clinical trials in areas that include pain management, behavioral interventions for sleep disorders and anxiety for dogs, inflammation reduction, and improvement in sleep patterns for cats (Kogan et al. 2015).

6.2 Demographics and Dog Owner Perceptions of Cannabis

In a follow-up questionnaire-based study to the 2015 publication cited above, Kogan et al. created a second online survey to assess US perception regarding hemp and marijuana products for their dogs. The survey originated from Colorado State University in collaboration with investigators at North Carolina State University. Survey participants were recruited via social media in late 2017. The data was collected anonymously. Dog owners residing in the United States were the only inclusion factors used in this study. Survey participants were asked for their demographics; the state they lived in was compared to states that currently have medical marijuana laws permitting legal use of marijuana for

medical and/or recreational purposes. Further questioning determined if they had given their pets a marijuana product or not and the reasons for use or nonuse of it. Finally, survey participants were asked for what reasons they had given the marijuana products to their pets.

A total of 1196 responses were collected, and after eliminating non-dog owners or non-US residents, there were 1068 responses. Eighty-four percent were female, the remainder, male. Eighty percent of respondents had used cannabis for their dogs. Those that did not use cannabis cited no medical reasons to do so or weren't aware that it could help. Pain relief, reduction of anxiety, and reduction of inflammation were the most common reasons for the use of cannabis in dogs in this study. Other reasons (total 36%) less commonly selected were for epilepsy (11.5%), cancer (9.4%), arthritis (1.9%), and allergies (1.3%)

The type of cannabis product most commonly used in the study were capsules or pills marketed for animals (57%), as compared to capsules or pills marketed for humans (3.9%). For edibles this same trend was seen, with more animal-labeled products being used in dogs than edibles labeled for people. No mention was made in this study of the most commonly used format which is the liquid oil infusion, also known as the "tincture." Side effects associated with the use of cannabis in dogs were reported only by a minority of dog owners (<5%), with the most common adverse effects being sedation, xerostomia, and associated polydipsia.

6.3 Safety of High-Dose Long-Term Exposure to CBD in Dogs

A study evaluating the safety of cannabidiol in the dog was published in the Fall of 2018. It was conducted at Colorado State University's College of Veterinary Medicine, Department of Neurology. The principal investigator, Dr. Stephanie McGrath, who is a veterinary neurologist and Assistant Professor at CSU's Veterinary Teaching Hospital, conducted a 6-week high-dose evaluation of the tolerability of two high doses of CBD in healthy beagle dogs. A sample population of 30 healthy beagle dogs were randomly assigned to receive 1 of 3 formulations: microencapsulated oil beads, CBD-infused oil, or CBD-infused transdermal cream for 6 weeks. Two dosage tiers were evaluated in this study, 10 mg/kg/day and 20 mg/kg/day. These dosages far exceed the dosages used in the two efficacy studies that followed this by a factor of 2× and 4× greater. The two efficacy studies evaluated the use of CBD for refractory epilepsy and osteoarthritis in the dog.

Complete blood counts, chemistry panels, urinalysis, and pre- and postprandial bile acids were performed at 0, 2, 4, and 6 weeks. Elevations in alkaline phosphatase double the high end of the reference range (140 IU/L) were observed in some dogs (11/30:36%) after being on the CBD for 4 weeks, although it did elevate in some dogs at 2 weeks, especially at the higher dosing tier. Long-term liver toxicity was not evaluated in this study, although bile acids and liver enzymes remained normal for all dogs throughout the study. None of the dogs receiving the transdermal formulation developed elevated alkaline phosphatase values. All dogs experienced mild diarrhea, although there was no correlation with formulation or dose. Six out of the 30 dogs developed vomiting, but there was no significant difference between the occurrence of vomiting and CBD dose or formulation.

Erythematous pinnae were the next most commonly reported clinical sign in this study. These otic changes were seen in 36% of dogs with the otic changes becoming more severe after 2 weeks in the 10 mg/kg/day dosage group for all three formulations. The transdermal cream had more incidences of otic changes than either the transmucosal or oral routes of administration, which is understandable since the transdermal crème was applied to the inside of the pinna. Less common findings included ocular discharge in 10/30 dogs (33%) and nasal discharge in 10/30 dogs (33%). Five dogs (17%) had salivary staining on their feet and occasionally on their ventral abdomen. Two dogs had spontaneous prolapsed glands of the nictitans. One dog had a transient elevated body temperature (104.2 °F). It was also observed that some dogs would salivate following administration of the CBD-infused oil formulations at both doses. The study concludes that CBD seems to be well tolerated in the dog at these high dosages but emphasizes that a larger and longer in duration safety study is needed to evaluate the very long-term effect of CBD on the liver and its association with diarrhea (McGrath et al. 2018).

6.4 Pharmacokinetics, Safety, and Clinical Efficacy of CBD Treatment in Osteoarthritic Dogs

The objectives of a recent Cornell study were to determine the oral pharmacokinetics and safety, as well as analgesic efficacy, of using CBD in dogs with osteoarthritis (OA). Single-dose pharmacokinetics were performed using two different doses of 2 mg/kg and 8 mg/kg of CBD in a carrier oil. From this data, a prospective, randomized, placebo-controlled, double-blind crossover study was conducted using 16 client-owned dogs with radiographically confirmed evidence of osteoarthritis who were enrolled and who completed this study. Dogs were randomized to receive either 2 mg/kg q 12 h orally of CBD oil or a placebo consisting of olive oil with a benign herbal extract at a similar volume q 12 h for 4 weeks. Subjects were given a 2-week washout period, and then the treatments were crossed over, and each subject received the other treatment twice daily for 4 weeks.

Veterinary assessment of lameness, movement, and response to manipulation, owner questionnaires (Canine Brief Pain Inventory (CBPI), Hudson activity scale), objective kinetic analysis on a pressure-sensitive walkway, hematology, and chemistry analysis were obtained at weeks 0, 2, and 4 for both oils. Statistical analysis was performed on the results, with a $p < 0.05$ considered to be significant.

Pharmacokinetics showed a half-life of elimination of 4–5 h at both doses and no observable side effects. Median maximum concentration of CBD oil was 102 ng/ml (61–132 ng/ml), and this peak was reached at 90 min following administration of the single dose of 2 mg/kg. The investigators on this study decided that since the pharmacokinetics of the 2 mg and 8 mg doses were so similar, they would use the lower of the two doses for the efficacy wing of this study.

Assessment of pain and mobility showed a significant decrease in pain and increase in activity ($p < 0.001$) at weeks 2 and 4 during CBD treatment as compared to baseline at each bi-weekly evaluation. It was found that the CBD oil resulted in reduced pain scores when compared to baseline on both bi-weekly examinations ($p = 0.03$). No side effects were reported by owners, but serum chemistry demonstrated an increase in serum alkaline phosphatase (9/16 dogs, 56%) while receiving the CBD oil, which reached significance at week 4 ($p < 0.005$).

The authors of this study conclude that the dogs with OA who received 2 mg/kg q 12 h were found to be more comfortable and active with very few undesirable side effects compared to placebo. The authors note that the CBD oil used in this study was a "strain-specific" extract and other products that do not have this same strain specificity may not have the same efficacy as measured for this proprietary product (Gamble et al. 2018).

6.5 Evaluation of Trends in Marijuana Toxicosis in Dogs Living in a State with Legalized Medical Marijuana: 125 Dogs (2005–2010)

This study correlated the number of medical marijuana licenses issued in the state of Colorado between 2005 and 2010 and the number of admissions to the ER for marijuana toxicosis. This is a retrospective case series from January 1, 2005, to October 1, 2010. A total of 125 client-owned dogs presented for known or suspected marijuana toxicosis with or without a urine drug screening test (UDST).

A significant correlation was found between the number of medical marijuana licenses and marijuana toxicosis cases seen in two veterinary hospitals in Colorado. Ingestion of baked goods made with high potency THC butter extractions resulted in two recorded deaths. The authors note that due to the difference in urine metabolites in the dog as compared to the human, the UDST test may not be valid in dogs. It is also important to note here that there have been no recorded deaths in the many hundreds of years of human use of marijuana. These two canine deaths were due to the toxicity of chocolate in dogs combined with the cardiovascular effects of THC on the myocardium. In each case, brownies or chocolate chip cookies were made with very large amounts of THC butter, and the two dogs, each rather small, ate very large quantities of the chocolate with cannabis. The authors were not able to attribute both deaths to the THC alone and felt there was a major influence of the chocolate contributing to their deaths (Meola et al. 2012).

6.6 Comparative Pharmacokinetic Study of Three Routes of Administration of CBD in the Beagle Dog

This study was designed to determine the pharmacokinetics of CBD in healthy dogs. A sample population of 30 healthy research dogs were assigned to receive 1 of 3 different formulations at a dose of 75 or 150 mg q12 h for 6 weeks. The dosage formats were (1) liquid oil infusion administered to the oral mucosa; (2) oral capsules with microencapsulated oil beads; and (3) transdermal application. Serial CBD plasma concentrations were measured over the first 12 hours and repeated at 2, 4, and 6 weeks. Greater plasma concentrations were measured with the oral CBD oil-infused formulation. The plasma half-life of CBD administered via this route after 75 mg and 150 mg doses, respectively, was 199.7 +/− 55.0 and 127.5 +/− 32.2 min. This study found that blood levels are dose proportional, as expected, and the oral liquid CBD absorbed transmucosally was the superior formulation of the three formulations tested, with orally administered microencapsulated beads the second-best formulation in terms of pharmacokinetic profile (Bartner et al. 2018).

6.7 Clinical Efficacy of CBD for Treating Osteoarthritis and Refractory Epilepsy in the Dog: A Pilot Study

The osteoarthritis (OA) wing of this efficacy study consisted of 24 client-owned dogs with clinical evidence of OA radiographically and who had an identifiable lameness. A double-blinded, randomized, placebo-controlled, study design was utilized, with each study group receiving medication for 6 weeks and a placebo for 6 weeks. The treatment group received 2.5 mg of CBD oil q 12 h. Gait analysis and an

activity monitor were used to gain objective data, and a behavioral questionnaire was given to the dog owners which provided subjective information. The study results for OA were not yet available at the time of this publication (McGrath 2018).

The epilepsy segment of the study consisted of 16 client-owned dogs who were diagnosed with idiopathic refractory epilepsy, having 2 or more breakthrough seizures per month while receiving conventional anticonvulsant therapies. Inclusion criteria included a normal neurologic exam and a normal epilepsy workup with an MRI and CSF analysis. Nine dogs were randomly assigned to the treatment group and seven to the control (placebo) group. The treatment group received 2.5 mg/kg CBD oil q 12 hours by mouth. The control group received placebo oil for 12 weeks. Study subjects were required to stay on their standard anticonvulsant drugs (AED). Routine blood work and CBD levels were determined every 4 weeks. AED levels were measured at the conclusion of the trial.

Sixty-seven percent (6/9) of the dogs in the treatment group experienced a greater than 40% reduction in average monthly seizures during the study, whereas only 29% (2/7) of the dogs in the control group had a greater than 40% reduction in average monthly seizures.

Elevations in alkaline phosphatase (ALP) were recorded for the treatment group and one dog in the control group. The single control dog had previously measured elevations in ALP, so this elevation was not considered to be relevant to the study. Six dogs (67%) in the treatment group had elevations in ALP measured at the end of the study. The mean ALP value was 619 IU/L (range 15–140 IU/L).

AED concentrations in the treatment group for phenobarbital decreased in 2/7 dogs (29%) and increased in 5/7 dogs (71%). In the control group, phenobarbital levels decreased in 3/5 dogs (60%) and increased in 2/5 dogs (40%); there was no significant change in either group. This is an interesting finding to note, because there has been a concern that CBD, which is metabolized through the P450 group of cytochromes, might interfere with the drug disposition of pharmaceuticals that also are metabolized through that pathway. From the results of this pilot study, that effect is not apparent, at least with respect to phenobarbital levels.

Potassium bromide (KBR) levels in the treatment group decreased in 2/3 dogs (67%) and increased in 1/3 dogs (33%). In the control group, KBR levels decreased in one out of two dogs (50%) and increased in one out of two dogs (50%). There was no significant change in either group, although the total number of study subjects was low in this pilot study. This research and the osteoarthritis section of this study have not yet been published, pending the results of the plasma analysis of cannabinoid levels that were measured at 0, 4, 8, and 12 weeks and the completion of the efficacy study of the effects of CBD on osteoarthritis.

The American Kennel Club Canine Health Foundation has granted nearly $400,000 in funding to this research group at CSU for a larger, expanded study with 60 dogs, as a result of the positive results of this pilot work, with respect to the use of CBD oil to address refractory epilepsy. This study will also be looking at uncontrolled epileptics having two or more seizures per month while receiving standard therapy. In this expanded study, which will use a crossover design, each subject will receive 12 weeks of treatment or placebo with a 4-week washout period between treatments. This study began in January 2018 and is currently enrolling patients (McGrath 2018).

7 Guidance on Veterinary Cannabis Products in the US Market

Cannabis preparations have historically been used for home remedies, as medicine, as a functional food, and as a source of nutrition, primarily for humans. However, there have been reports of cannabis products being used in animals dating back to the 1800s, when cannabis had become a popular herbal remedy at a time prior to the development of the pharmaceutical industry. Patent medicines containing cannabis, usually in an alcohol tincture, were sold to horse owners for colic and other equine ailments, and topical liniments were used externally for joint and lameness problems.

As cannabis became vilified and stigmatized in the 1930s, and as pharmaceutical medicines became more available as substitutes for cannabis products, the use of cannabis in the horse became less common to nonexistent. For our companion animal species, dogs, cats, and horses, the use of cannabis products did not gain popularity until the state-by-state legalization of cannabis for medicinal purposes began in the mid-1990s. Pet owners, learning of the many benefits that cannabis has created for people, naturally began to explore its use in their pets for the medical problems that were not easily solved by conventional veterinary medicine. These are problems such as epilepsy that are resistant to pharmaceutical remediation; pain that is poorly responsive to opiates, steroids, and nonsteroidal anti-inflammatory drugs; cancer treatment side effects such as nausea and vomiting; and to treat cancer. Some veterinary patients respond well to NSAIDs for osteoarthritis, but the drugs themselves can cause toxicity in sensitive patients. The use of cannabis as an alternative means of remediating the pain of arthritis can substitute for these drugs or allow for lower and safer dosages of the pharmaceuticals. Several veterinary randomized placebo-controlled clinical trials that have been conducted have demonstrated objectively the benefit of cannabis for osteoarthritic pain in the dog (McGrath 2018; Gamble et al. 2018).

7.1 Cannabis Product Formats and Delivery Methods

There are several formats of cannabis products for companion animals, currently in the marketplace, and several up-and-coming formats currently undergoing research and development efforts by forward-thinking companies. The route of administration is a primary contributing factor in the effectiveness of any medicine, natural or otherwise. Choosing the appropriate delivery method for the patient can greatly affect medical outcomes. The associated bioavailability of the drug should be balanced with the practical needs of the patient.

Dogs have proven to be highly sensitive to the adverse neurological effects of the psychoactive decarboxylated THC. They can benefit from the use of the non-decarboxylated non-psychoactive THCA, without any fear of a visit to the veterinary ER. THCA, although not psychoactive, is still a Schedule I controlled substance according to the Drug Enforcement Agency. Although the THCA does not bind to CB_1 receptors in the brain, which is why it is psychoactive, it is regulated through many other entourage non-cannabinoid receptor-mediated pathways.

Juicing of cannabis leaves is gaining popularity as a method of preparation and consumption. The cold extraction of raw cannabis preserves the heat-sensitive acidic cannabinoids THCA and CBDA that readily undergo degradation through the decarboxylation pathway. Decarboxylation typically occurs at temperatures above 100 °C that are common for combustion (smoking), vaporization, or hot extraction. THCA has a significantly lower affinity for the endogenous cannabinoid receptor CB_1 and does not have the same psychoactive effects as preparations containing "activated" of "decarboxylated" THC. Consumption of unheated cannabis preparations allows for the administration of significantly higher doses of acidic cannabinoids, without the psychoactive effects of THC. Juicing of raw cannabis would have the advantage of retaining volatile terpenes, which also have important medicinal properties. Original terpene profiles are generally altered in extraction methods involving heat.

7.1.1 Oral Route of Administration

Ingested route is very common and still quite effective, in spite of its poor bioavailability. Pharmacokinetic (pK) studies documenting the bioavailability of oral cannabis extracts, specifically the major cannabinoids THC and CBD in both humans and dogs, demonstrate that only 5–10% of the lipophilic drug reaches its intended active site. In spite of this relatively poor bioavailability, good clinical responses have been documented in both species. pK studies documenting the bioavailability of cannabinoids in the equine species remain to be performed, as well as in other veterinary species. The majority of products currently available for animal species are targeted toward ingestible formulated products. Some examples of these formulations are highlighted below.

Capsules—The first animal companion cannabis product to be launched in the United States, shortly after the federal legalization of low THC cannabis ("hemp"), contained powdered hemp seed meal infused with the lipophilic extracts of the cannabis plant and was manufactured into capsules. Capsules contain a fixed volume of active materials and are limited in efficacy to specific weight ranges of patients. This limitation necessitates manufacturing and packaging multiple capsule sizes to accommodate the many different sizes of veterinary species, Chihuahua to Great Dane to draft horse. Capsules are convenient to keep and administer and are a dosage-form that is familiar to most consumers. They can be hidden in a small amount of food to facilitate absorption on an empty stomach, but they are also subjected to rapid first pass liver metabolism and, based on the published pK study from Colorado State University, are less bioavailable than a lipophilic liquid absorbed through the oral mucosa (Bartner et al. 2018).

Tinctures—The second cannabis product format introduced to the US animal health market was a product that has become commonly known in the cannabis industry as a "tincture." These are oil infusions of lipophilic extracts of the cannabis plant and can contain either high THC cannabis ("marijuana") or low THC cannabis ("hemp"). Carrier oils can be any oil, but most commonly grape seed oil, hemp seed oil, and medium-chain triglycerides are used in these formulations. It's worth mentioning that the word "tincture" was derived from botanical medicine and in that context denotes an ethanolic extract of a botanical material.

Tinctures can contain just cannabis or can be compounded with additional terpenes and other botanicals and nutraceuticals or even pharmaceuticals for a more targeted effect. Tincture labels should include the potency of the formulation in total milligrams of CBD and THC and other measurable levels of cannabinoids, as well as listing the other amounts of the active ingredients in the bottle, and any preservatives that have been added to improve shelf life. Tinctures have the advantage of being scalable in terms of being able to dose different sizes of animals, since all that is needed for a larger or smaller patient would be more or less volume, respectively. Tinctures can also be added or mixed with a small amount of tasty food to facilitate administration but are most efficiently absorbed transmucosally from the oral cavity.

A pharmacokinetic study comparing the bioavailability of transmucosal, oral, and transdermal routes of administration for CBD was recently completed (Bartner et al. 2018). It was

found that transmucosal administration of a lipophilic liquid produced the highest C_{max} values in this study. Thus, the oil-based proprietary tincture in this study, applied transmucosally to the oral mucous membranes, gums, inner lips, tongue, and buccal pouch, would be the most bioavailable means of administration compared to the other two materials evaluated in this study.

Extended stability studies of cannabis products are needed to determine the shelf life of the formulation, which generally ranges from 12 to 24 months. This "outdating" should be expressed on the label as a manufacturing date or a "best by" date so as to inform the consumer of how recently the product has been manufactured. This dating of the formulation guides the consumer in selecting a product where its potency should meet label claims based on its aging over time and the subsequent gradual deterioration of the delicate active materials contained therein. For a patient who doesn't need much extract on a daily basis, a bottle containing a large amount of material could conceivably expire before it is finished.

Dosage-form animal health products are commonly used to administer pharmaceuticals and nutraceuticals. Treats that contain nutraceuticals or pharmaceuticals, aka "dosage-form treats," have become very popular among pet-owning consumers. Dosage-form treats need to have the intent, and be labeled, as nutraceuticals, not as nutritional compounds. Neither cannabis nor hemp are approved feed ingredients by the FDA-CVM and AAFCO. This means that the guaranteed analysis (GA) and a nutritional statement on the label and/or product package with total calories and calories from fat is not appropriate for a dosage-form treat and could cause a "stop sale" letter to be issued by the FDA-CVM for selling an adulterated nutritional product.

The label of dosage-form treats, based on NASC and FDA-CVM guidelines, needs to contain a listing in the order of decreasing weights of the materials that are the "active ingredients" and the "inactive ingredients." This second category would include the nutritional components of the treat on the label.

Hard biscuits and soft-chews currently are the most popular dosage-form treats available in the marketplace. Like capsules, these products can contain only a fixed amount of the active ingredients, thus limiting the weight ranges addressed by a single dosage-form treat size. The advantages of dosage-form treats are the relative ease of their administration. The potential disadvantages could be patient hypersensitivity to the ingredients in the treat, the need for multiple treats at a time to treat a larger weight patient, the reduced shelf life, and the potential that the manufacturing process that involves baking or heating and extruding the material could cause heat adulteration of the product. Postproduction product analyses are critical to ensure that product potency has not been compromised by the manufacturing process.

Pellets and powders are scalable for dosing a wide variety of target species weights. There are a few powders made from low THC cannabis ("hemp") available. These powders can be manufactured from the powdered plant material or from the pharmaceutical modification of the lipophilic extract of the plant material into a powder format or may use an inert powder that is infused with the lipophilic extract of the cannabis plant. For most of the applications for animals, the low THC variety of cannabis is the preferred raw material for manufacturing this powder due to the potential for problems with high THC cannabis both in regard to the patient response and as regards the stricter regulatory environment for high THC cannabis.

Powders can be contained in wide-mouthed bottles with a measuring scoop in them that can be used to estimate effective dosages. Standard kitchen measuring spoons can also be used to scoop out the appropriate dosage for the size and condition of the patient. Powders can be packaged into stand-up pouches, as well as sticks, sachets, and, of course, capsules. Individual serving sizes make sticks, sachets, and capsules more convenient for dosing but remove the scalability benefit of powder over dosage-form treats.

Pellets are pressed from dried cannabis plant material, most commonly using the low THC cannabis variety ("hemp"). These pellets can be given to horses, goats, sheep, and other farm animals, as well as poultry, swine, zoo animals, caged birds, and pocket pets. A big concern with giving horses cannabis, even low THC cannabis, is that there would be enough THC even in low THC cannabis that the horse would test positive for THC on drug testing and be disqualified from whatever event it was being tested for.

Water-soluble cannabinoids apply existing technology using lipid emulsifiers and reducing the size of the oil droplets to as small a size as possible, usually in the range of nanometers. Water solubility increases the plasma concentrations and helps the lipophilic drug reach the active site. Further, a rapid onset is observed with water-soluble formulations that do not have the same long-lasting effects. Although published pK studies comparing the bioavailability of lipophilic versus hydrophilic preparations of cannabinoids administered through the same route do not exist, there are credible pK studies using other lipophilic materials, such as Coenzyme Q10, which document the improved bioavailability to be as much as 5–8 times better than the lipophilic material with this approach. The impact of increased bioavailability on clinical outcomes remains to be measured as these water-soluble cannabinoids become more available; however, less cannabinoids are required in water-soluble formulations to achieve similar active site concentrations compared to oil-based solutions.

Transmucosal routes of administration can be very effective in most contexts, as it is fairly easy to apply the liquid cannabinoids to the oral mucous membranes. Bartner

measured the comparative bioavailability of transmucosal with oral and transdermal routes in 30 beagle dogs. The transdermal method of administration was 12–32% as bioavailable as the transmucosal approach between 75 mg and 150 mg/day, based on the C_{max} values. The oral approach was 55–68% as bioavailable as the transmucosal approach. It was concluded from this study that the most bioavailable administration avenue among these three dosage formats was the transmucosal approach (Bartner et al. 2018).

7.1.2 Topical Administration

Topical applications of cannabinoids have low systemic bioavailability but will penetrate locally to benefit the regional anatomy. For most veterinary species that have hair or feathers covering their bodies, "salves" and "balms" that are oil-based run the risk of matting the hair and interfering with the application to the epidermis where it will be absorbed locally. Products that are in solvents like alcohol or use emulsifiers to create a water-soluble cream or lotion have a better chance of penetrating the hair or feathers and ultimately penetrate the skin. These are termed "liniments" if they contain alcohol and "creams" or "lotions" if they are emulsified and water soluble.

Transdermal applications involve the use of a bipolar material such as a phospholipid to carry the cannabinoids into the local circulation and from that enter the systemic circulation. Transdermals can be creams or can be in "patches" that use a membrane to separate the transdermal solution from the skin. For veterinary species, transdermal liquids and patches will need to have a spot of hair clipped prior to application. Some anatomical sites that are hairless, such as the inside of the pinna and the ventral inguinal regions, do not need clipping and facilitate administration. It is recommended prior to reapplication of the transdermal cream to cleanse the site of application to improve absorption. Administration to the inside of the ear flap prevents the veterinary patient from licking the transdermal cream from an accessible area such as the inguinal region. Transdermal medication absorption is dependent upon the amount of local vascularity to carry the medication from the skin into the systemic circulation.

7.1.3 Inhalation

Inhalation is an impractical delivery method for veterinary species, except maybe primates or laboratory animals connected to breathing masks. As water-soluble versions of cannabinoids are developed, the possibility exists that a nebulization method could be employed to allow for absorption through the large surface area of the lungs. Inhalation methods are rapid onset and bypass first pass metabolism in the liver.

7.2 Zero-THC Hemp Extracts

Although low THC cannabis, which is called "hemp," already has less than 0.3% THC on a dry matter basis at the time of harvest, there are a number of situations where significantly reducing the amount of THC to undetectable levels has distinct advantages for veterinary species. Dogs are more sensitive to the adverse neurological effects of THC when they are naïve to exposure and have not developed tolerance. Some dogs are more sensitive than others, and anecdotal reports from veterinary clients (personal communication) indicate that there is a small percentage of dogs who react adversely to the small amount of THC present in hemp cultivars. For these dogs a zero-THC product would be advantageous.

Horses can have unpredictable responses to THC, potentially increasing the risk to the horse, the horse owner, and the veterinarian. Additionally, both horses and dogs in competitive events are commonly drug tested in order to qualify to compete. The use of a zero-THC product would make it less likely the animal would fail the drug test due to detectable levels of THC. Drug testing laboratories commonly test both urine and blood for many substances, including THC and CBD. Most venues will disqualify if any THC is detected (zero tolerance), but many do not disqualify for the detection of CBD. It is up to the individual event to decide what the disqualifying parameters are. The World Anti-Doping Agency (www.WADA-ama.org) has allowed the use of CBD in athletes since January 2018. It still disqualifies for THC, synthetic cannabinoids, and cannabimimetic agents. For this reason, Olympic athletes that are using a zero-THC source of CBD will not be disqualified, per the example of gold medal winner, Michael Phelps.

The veterinarian co-author of this chapter has distributed over 40,000 bottles of zero-THC tincture to veterinarians over the past 2 years. Reports from hundreds of these veterinarians have documented the clinical strength of the zero-THC formulation to reduce pain, improve mobility, bring some tumors into remission, and successfully address uncomplicated epilepsy, type 2 diabetes, and anxiety-related behavior problems such as noise phobias.

Prior to the discovery and use of all of the many non-THC cannabinoids in hemp, it was thought that THC was absolutely necessary for any clinical benefits. It is now known from these many cases that have successfully been treated with zero-THC extracts that the entourage effect does not need THC to be activated. In patients with reduced endocannabinoid tone, it may be necessary to use a small amount of THC to replace the endogenous ligand for better clinical response. Cases of severe pain and aggressive cancers may also benefit from THC, but for most other clinical cases, THC is not necessary.

7.3 Dosing Considerations and Strategies for Veterinary Species

The determination of evidence-based dosages through dosage-tiered Phase I studies in any veterinary species has yet to be conducted. Several of the randomized clinical trials recently published from veterinary academic institutions have used dosages extrapolated from published studies in human and laboratory animals, verified by single-dose pharmacokinetics to ensure detectable blood levels over a therapeutic period of time. These veterinary studies in dogs used a single dosage, which is quite high, but these high dosages did have statistically significant results with clinical improvement in response to clinical patients with OA and refractory epilepsy. However, there have not been any incremental dose-to-response studies performed to see if a lower dose might also benefit veterinary patients and be more cost-effective for the pet-owning public.

Dosages in the human patient have been derived both from published objective studies and from the clinical impressions of hundreds of physicians who have been trained in medical cannabis in the 29 states with medical cannabis legislation since 1996 when cannabis was first legalized for medical purposes in the United States (MacCallum and Russo 2018). Due to the legal considerations affecting veterinarians' clinical use of cannabis, there has not been this kind of hands-on practical empirical dosage-setting by veterinary clinicians. Instead, dosages have been empirically and anecdotally determined largely by pet owners and by a few veterinarians willing to risk their veterinary licenses to employ cannabinoids with their patients.

The data from veterinarians who have been recommending phytocannabinoids for their patients shows that veterinary species have a "biphasic response" to cannabis dosages in the same way that humans do. "Biphasic" means that a low dose (or "microdose") will generate one set of effects and address one set of clinical issues and a high dose (or "macrodose") will produce a second set of effects and address a second set of clinical issues.

Microdoses are considered to be less than 0.5 mg/kg BID of cannabinoids. Macrodoses would be greater than 2.0 mg/kg BID. Individual responses to dosage levels may vary. Based on anecdotal reports by veterinarians and pet owners who are using microdoses, the effective dosages used in dogs and cats have been reported to be as much as 40 times less than the macrodoses used in the CSU safety study or 5 times less than those used in the CSU efficacy study. There will be a small portion of animals that will develop the reported side effects of diarrhea, sedation, or elevated serum alkaline phosphatase, even with these lower doses.

McGrath evaluated the safety of administering high doses of CBD over a 6-week period. The results found that at the macrodose level (10–20 mg/kg/day), there were a greater frequency of adverse reactions to hemp extracts. Diarrhea, sedation, and serum alkaline phosphatase elevations were recorded in the subjects receiving 10 and 20 mg/kg/day (McGrath et al. 2018).

Tischler in his presentation at the Second Annual Conference at the Institute of Cannabis Research at Colorado State University in Pueblo discussed the value of microdoses in the human patient over the use of macrodoses. Microdosing serves to reduce or eliminate the psychotropic effect of THC through sub-psychotropic dosing and through the development of tolerance over a period of 2 weeks. Veterinary patients, for whom the use of THC is necessary and therapeutic (severe pain, appetite stimulation not effective with CBD, certain types of neoplasia), using the microdose strategy initially will facilitate the development of tolerance to the adverse effects of THC. Once this is achieved, THC dosages can be escalated to achieve the desired clinical benefit(s) (Tischler 2018).

In an unpublished study, the veterinarian co-author of this text gave 30 horses, in 3 different stables, dosages of 25–50 mg of CBD in a zero-THC hemp extract once or twice daily to address complaints of anxiety, gait abnormalities, mild to severe laminitis, and metabolic syndrome. Study subjects averaged around 1000 pounds. It was found that for anxiety and mild cases of lameness or gait abnormalities, administration of 25 mg once or twice daily was adequate to elicit a response with regard to anxiety from loading up into a trailer, or at events, or for mild gait abnormalities. In one stable the horses were only able to be given their dose once daily, yet that single dose still produced good clinical results.

For more severe conditions such as moderate laminitis, other sources of lameness, or metabolic syndrome, it was found that 50 mg once or twice daily was sufficient for clinical response. When horse owners were asked to discontinue giving the hemp extracts so as to determine withdrawal times for CBD, for situations in which the horses may be drug tested for an event, many refused to stop administration of the hemp, as they were very pleased with their horses' response to the hemp extracts. Horses have evolved to be very efficient in absorbing fats from their diet, as their natural diet of forage is very low in fats and oils. pK studies in the equine are very much needed to better understand dosing intervals and levels.

For most other animals, we use the dosage range established empirically through thousands of veterinary uses, starting with a low dosage and slowly increasing the dose over time. It is recommended to give a dose for 10–14 days to allow for the upregulation of CB receptors, and if after 2 weeks patient clinical response is inadequate, then increasing the dosage twofold greater than the starting dose may produce a better response in a given patient. Following through with dosage escalation, slowly over time, will yield the best clinical results. "Start low, go slow, stay low" is the mantra for dosing a patient whether human or veterinary,

and this protocol, combined with the safety and efficacy studies of McGrath, allows the practitioner to be able to provide a dosage as high as 20 mg/kg/day safely (MacCallum and Russo 2018; McGrath et al. 2018).

7.3.1 Ratio Dosing

Ratio dosing is the use of cannabis formulations that contain both THC and CBD in specific proportions to each other. The goal of this approach is twofold:

1. It allows for the development of tolerance to THC through the initial use of a high CBD to low THC ratio (THC:CBD/1:25) to avoid psychoactivity while tolerance to the adverse effects of THC is being established.
2. Once tolerance has been established, the dosage of the THC fraction in this combination formula can then be escalated safely. Escalation of the THC dose will also escalate the CBD dose administered, which may be the desired approach for a given patient's clinical needs.

If it is determined that a patient needs a higher amount of THC to provide better medical benefits but may not need as much CBD as escalating the THC dose in a low ratio formula would provide, then moving to a ratio formulation with an equal amount of THC:CBD may be more efficacious. Equivalent ratio formulations of THC:CBD (1:1) are being used empirically and effectively for moderate to severe pain and in oncology patients for whom the low THC ratio product does not provide sufficient antineoplastic activity or remediation of oncologic side effects from therapeutics or the disease itself.

In rare situations in veterinary species, it may be necessary to provide a high THC formulation for improved pain management or to better address the needs of the oncology patient. THC:CBD/4:1 is typically the ratio that is being used clinically. The need and use of this in veterinary patients has a higher potential for adverse effects than the lower THC ratio formulations. In all cases of using THC in veterinary species, it is important to develop tolerance first by using the low THC or THC-free products mentioned previously for the first 2 weeks of therapy to avoid adverse reactions or trips to the veterinary ER.

7.3.2 Adverse Events (AEs) and Dosage

McGrath reported that the CBD safety study determined that a dosage of 10 or 20 mg/kg/day of a full-spectrum CBD divided BID produced a greater incidence of adverse events than reported at lower dosages. Diarrhea and sedation were the two dominant AEs. Elevation of serum alkaline phosphatase levels was also observed to be significant events at these higher dosages. Other liver function tests, such as bile acids and ALT, were within normal limits (McGrath et al. 2018).

Sellers et al. (2013) found that the use of a 1:1 ratio of THC:CBD in their sublingual spray, Sativex™, was associated with significantly less AEs than the 100% pure synthetic THC capsule, Marinol™. This illustrates the harm reduction that comes with adding CBD to a formulation containing THC or a synthetic THC (Sellers et al. 2013).

Russo reported that the incidence of AE associated with the drug Sativex™, approved throughout the world for muscle spasticity with MS, was significantly less when the drug was gradually titrated to the patient's response. This study found that most AEs will be early and transient using a modification of the dosing strategy by starting at a lower dose increasing it slowly to achieve to clinical effect (Russo et al. 2011). In a later paper, MacCallum and Russo state, "Cannabis medicine doses must be individually determined, as (the effective dosage for a specific patient) . . .. depends on the endocannabinoid tone of that patient" (MacCallum and Russo 2018).

7.3.3 Drug Interactions with Cannabis

It is reported that in human medical marijuana patients, physicians have only rarely observed clinically significant drug interactions. MacCallum and Russo (2018) state, "there is no drug that cannot be used with cannabis, if necessary" (MacCallum and Russo 2018). Depending on the strength of the affinity of the metabolite for the metabolizing cytochrome, serum levels of cannabinoids or pharmaceuticals may increase with inhibitors or decrease with enzyme inducers. It is known that THC and CBD in human are oxidized by the p450 cytochromes (CYP2C9, 2C19, and 3A4). It is assumed that this is similar to the metabolic pathways in the dog and other veterinary species, but studies still need to be conducted in each species to detail possible interspecies variation.

In human patients the main AEs have been associated with the concomitant use of other CNS depressants with cannabis. High-dose CBD has been found in human to interfere with the metabolism of clobazam, thus potentially requiring a dose reduction of that drug when used with CBD (Devinsky et al. 2017). Drug interaction studies for each individual drug and cannabinoid are lacking, but the studies that currently exist have found no increased toxicity or loss of effect with concomitant medications. It is good medical practice, however, to continue to test for these interactions as individual responses may not be the same or predictable from population responses.

McGrath in her refractory epilepsy study measured both post-pill phenobarbital levels and potassium bromide (KBR) levels in her study subjects at the end of the 6-week study period. She found no statistical difference between the treatment and placebo groups for either anticonvulsant. This may indicate that, in spite of the theoretical potential for herb-drug interaction between CBD and P450-metabolized drugs, at

least in dogs on anticonvulsants, the interference may not be clinically relevant. Regardless, it is good medical practice to retest important serum drug levels 2 weeks following initiation of CBD therapy, especially if using macrodose protocols. McGrath mentioned the small study group size in this pilot work as possibly not giving an accurate indication of whether there really is herb-drug interaction or not, and she mentioned that she will analyze these levels not just at the end of her AKC-funded clinical trial but at regular intervals during the trial (McGrath 2018).

8 Current Good Manufacturing Practices (cGMP)

The National Animal Supplement Council (NASC.cc) in partnership with the FDA-CVM has established cGMP guidelines for animal nutraceuticals. These have recently been updated to include the recently enacted FDA Food Safety Modernization Act (FSMA). The NASC will provide third-party inspections of manufacturing facilities of its members to ensure that they meet cGMP standards. NASC member companies that pass their audits successfully can display the NASC seal on the product labels which can guide the consumer to locate products that meet the highest standards of quality and transparency.

Product labels need to contain information about the batch or lot number of the manufacturing process, outdating, company contact information, precautions and contraindications, and the intended use of the product, which, as a nutraceutical, cannot have any medical claims made in the label copy or marketing material or even in the name of the product. For instance, a product that contains the letters CBD, or the word "cannabidiol" in its name or on the label, runs the risk of enforcement by the FDA. This is because the FDA has recently approved CBD as a drug to treat resistant pediatric epilepsy. Medical claims are a common reason for FDA-CVM stop sale letters.

8.1 Third-Party Lab Testing

Quality control testing is a critical aspect of any manufacturing process. Without QC testing, manufacturers would not be able to manufacture products with standardized concentrations.

In addition, contamination can be introduced, and the only way to assure that the product remains unadulterated is through validated testing methods. Other important things to consider include organic cultivation methods, Good Agricultural Practices (GAP), and cGMP protocols.

The analytical laboratory performing the analysis must be validated for accuracy and precision in its measurements in order for the standardization of product potency to be effective. Companies that use in-house laboratories must have those results validated through the use of validated third-party analytical laboratories. It is also up to the labs to apply statistically valid sampling methods to obtain a representative sample of a batch in order to generate meaningful data.

There are a few major concerns that third-party testing can alleviate:

Cannabinoid and Terpene Potency Testing—This is absolutely essential in order to achieve effective and consistent dosing of a patient. An accurate analysis for each manufactured batch is needed to meet strict food and drug guidelines. The contents need to be listed on the product label and in marketing materials to allow the clinician to more accurately establish an effective dosing strategy for the patient. Further, it allows the pet-owning consumer to better select the appropriate potency of product for their size of animal and the potential duration of therapy needed for that product. Manufacturers can make label claims regarding the cannabinoid and terpene content of their products in order to mislead consumers and inflate perceived value of their products. This can pose a serious health risk if a patient is not getting the required dosage necessary to alleviate their conditions. Certificates of analysis on cannabis-derived products convey the potency and potential dosages of products.

Pesticide Contamination—The application of pesticides has long been a standard practice in cultivation of cannabis plants, despite market demand for organic products. The pesticides that are commonly sold in hydroponic stores can present a specific health risk to both animals and humans. For example, a commonly sold fungicide called Eagle 20 has a principal active ingredient called myclobutanil, a compound that when exposed to heat can generate hydrogen cyanide as a reactive by-product. Another fungicide and plant growth regulator called paclobutrazol has been shown to cause downstream kidney and liver damage in animal case studies, and the state of California has designated it as a Category I pesticide, meaning absolutely no detectable amount is allowed in a cannabis product. Be sure to ask for a robust multiresidue pesticide analysis from a third-party laboratory for any organic cannabis products.

Microbiological Contamination—Food testing has long set the standard for ensuring microorganisms are not present in products meant for consumption, but certain cannabis operations have yet to fully mitigate this risk. While it is fairly rare to find organisms like *E.coli* or *Salmonella* in cannabis, it is very common to find *Aspergillus*, a certain type of mold that is very prevalent in outdoor cannabis. *Aspergillus* poses a very specific health risk, especially for patients with a compromised immune system. This is due to a type of neurotoxic by-product produced in the spores collectively referred to as mycotoxins. If the veterinary patient has

any type of immune disorder, it is imperative that any products purchased come with a certificate of analysis certifying the absence of microorganisms.

Heavy Metal Contamination—Cannabis is known to be a bioaccumulator, meaning it will absorb contaminants from the soil. While this can be useful in some applications such as remediating poor soils, it can cause a huge risk if the hemp or cannabis plant was grown in contaminated soils. Arsenic, cadmium, lead, and mercury are the principal dangers, and heavy metal testing should be conducted on any plant-based products to ensure a clean product. Be especially wary if the plants were grown in a country with poor environmental regulations.

9 Concluding Remarks and Future Directions

Veterinary medicine has not seen the same advances compared to human medicine for objective, non-biased scientific evidence for the use of medical cannabis in veterinary species. This is due, in part, to the fact that the legalization statutes, state by state, do not provide for similar legal privileges for veterinarians and their patients as physicians have for recommending cannabis for their human patients.

Nonetheless, there are now several university-based, prospective, double-blinded, placebo-controlled, randomized clinical studies examining CBD from hemp extracts to measure its safety and pharmacokinetics. Included with these studies, once safety has been confirmed, is measuring the efficacy of hemp oil extracts over diagnosed veterinary conditions such as osteoarthritis and epilepsy. Some of these studies have already been published, some have been presented at professional conferences, and some are in press. These landmark studies have all been reported and summarized in this chapter.

Due to the nearly universal distribution of the endocannabinoid system in all chordates, and in many invertebrates, the same or similar benefits of cannabinoids found in humans also can be applied to most veterinary species. This chapter highlights what we know about cannabinoids and their interactions with the endocannabinoid system in animals. A thorough review of the scientific literature has been compiled here for veterinarians and veterinary scientists to better understand this fascinating and emerging therapy and, by understanding it, to be better able to deploy cannabinoid therapies for their patients and formulate more effective cannabinoid medications.

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