

1 Review

## 2 The Endocannabinoid System of Animals

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5 **Simple Summary:** The recent discovery of the Endocannabinoid System, and its ubiquitous  
6 presence in nearly all animals, has opened the door to novel approaches targeting pain  
7 management, cancer therapeutics, modulation of neurologic disorders, stress reduction, anxiety  
8 management and inflammatory diseases. Endocannabinoid-related molecules, both endogenous  
9 and exogenous, are able to function as direct ligands or otherwise influence the ECS. This review  
10 article introduces the reader to the endocannabinoid system in animals, and documents its  
11 potential as a source for emerging therapeutics.

12 **Abstract:** The endocannabinoid system has been found to be pervasive in mammalian species. It  
13 has also been described in invertebrate species primitive as the Hydra. Insects apparently are  
14 devoid of this otherwise ubiquitous system that provides homeostatic balance to the nervous and  
15 immune systems, as well as many other organ systems. The endocannabinoid system (ECS) has  
16 been defined to consist of three parts: 1. Endogenous ligands, 2. G-protein coupled receptors  
17 (GPCRs), and 3. Enzymes to degrade and recycle the ligands. Two endogenous molecules have  
18 been identified as ligands in the ECS to date. These are the endocannabinoids: Anandamide  
19 (arachidonoyl ethanolamide) and 2-AG (2-arachidonoyl glycerol). Two G-coupled protein  
20 receptors have been described as part of this system, with other putative GPC being considered.  
21 Coincidentally, the phytochemicals produced in large quantities by the *Cannabis sativa* L plant, and  
22 in lesser amounts by other plants, can interact with this system as ligands. These plant-based  
23 cannabinoids are termed, phytocannabinoids. The precise determination of the distribution of  
24 cannabinoid receptors in animal species is an ongoing project, with the canine cannabinoid  
25 receptor distribution currently receiving the most interest in non-human animals.

26 **Keywords:** Endocannabinoid system; Anandamide; 2-AG; Cannabis; Cannabinoid Receptor 1;  
27 Cannabinoid Receptor 2; PPARS a, b; Ht1a; TRPV1; GPR55; cannabidiol; CBD; THC; CBG; CBC;  
28 tetrahydrocannabinol

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## 30 1. Introduction

31 Common to nearly all animals except the Phyla *Protozoa* and *Insecta*, the endocannabinoid system  
32 arose in the phylogeny concurrently with the development of the nervous system as multicellular  
33 animals developed increasing complexity. This system was unknown to scientists until the  
34 mid-1990's, but research into this fascinating and clinically useful system is advancing rapidly,  
35 especially with the use of state-of- the-art LC-MS analyzers, and immunohistochemical and  
36 polymerase chain reaction (PCR) analytic technologies.

37

38 For over 70 years scientists have been hobbled by the legal and regulatory prohibitions related to  
39 research into cannabis and its associated molecules. Phytocannabinoids from *Cannabis sativa* L. and  
40 the naturally occurring endocannabinoids can serve as ligands in the ECS.

41

## 42 2. The Endocannabinoid System

43 The endocannabinoid system (ECS) has been identified in nearly all animals, from complex  
44 mammals like primates to phylogenetically primitive animals such as the cnidarians. The near  
45 universal presence and early emergence of the ECS, evolutionarily, is a strong indicator of its  
46 biological importance. Cannabinoid receptors are expressed in most animals, including vertebrates  
47 (mammals, birds, reptiles, and fish) and invertebrates (sea urchins, leeches, mussels, nematodes, and  
48 others).

49 The most primitive animal with an ECS is the Hydra (*H. vulgaris*), a cnidarian in the class Hydrozoa,  
50 which is the first animal to develop a neural network. A study of the ECS in the Hydra determined

51 that the major function of the ECS in this primitive organism is to control its feeding response [1]. It  
52 is evident from this data that all veterinary species contain an ECS. Therefore, an understanding of  
53 the ECS in these species is critical to the development of clinical applications for endocannabinoids  
54 and the phytocannabinoids, terpenes, and flavonoids derived primarily from *Cannabis sativa* L.

55 The ECS was discovered during the search for the biological targets for the recently described  
56 structure of the psychotropic phytocannabinoid,  $\Delta$ -9-tetrahydrocannabinol (THC) [2]. THC is the  
57 only psychotropic cannabinoid found in *Cannabis sativa* L and is responsible for some of its  
58 biomedical activity along with the non-psychotropic cannabinoids such as cannabidiol (CBD),  
59 Cannabigerol (CBG), Cannabichromene (CBC), other minor cannabinoids, terpenes and  
60 flavonoids.

61 Research sponsored by the National Institute on Drug Abuse in Bethesda, MD led to the discovery of  
62 the GPCR which was named CB<sub>1</sub>. Two years following the discovery of the CB<sub>1</sub> receptor,  
63 Makriyannis characterized a second GPCR named CB<sub>2</sub> [3]. Both the CB<sub>1</sub> and the CB<sub>2</sub> receptors play  
64 important roles in many essential biological processes, including neuronal plasticity, pain, anxiety,  
65 inflammation in general and especially neuroinflammation, immune function, metabolic regulation,  
66 reward, craving and bone growth [4].

67 Following the discovery of the membrane receptors that accept plant-based ligands, researchers  
68 quickly identified the endogenous ligands that bind to the cannabinoid receptors (CBR). These  
69 endogenous ligands are the endocannabinoids (eCB), arachidonoyl ethanolamide (AEA), a  
70 long-chain fatty acid amide, was described in 1992, and was named: "Anandamide" by Mechoulam,  
71 after the Sanskrit "Ananda" meaning bliss, as this endogenous molecule is responsible for feelings of  
72 well-being. The ester of this fatty acid amide, 2-arachidonoyl glycerol (2-AG), was discovered  
73 shortly thereafter in 1995 [5-7]. Both of these compounds are able to activate both CB<sub>1</sub> and CB<sub>2</sub>  
74 receptors, and were found to produce effects similar to THC, which is the only phytocannabinoid  
75 that binds orthosterically to the cannabinoid receptors.

76 eCBs are produced ad hoc by enzymes within the cell membrane that are activated by calcium ion  
77 elevation. The level of endocannabinoids is termed the “Endocannabinoid tone” and that level varies  
78 based on the specific tissue in which they are found. These levels are dependent upon their rate of  
79 production minus the rate of their enzymatic degradation by another set of enzymes, fatty acid  
80 amide hydrolase (FAAH) and monoacyl-glycerol lipase (MAGL). Respectively, these enzymes  
81 deactivate AEA and 2-AG.

82 By definition, the endocannabinoid system consists of these three parts:

- 83 1. Endogenous ligands
- 84 2. Membrane receptors
- 85 3. Deactivating enzymes

86

87 The regulation of endocannabinoid tone is modulated by a transport mechanism that carries  
88 released eCBs retrograde from the post-synaptic cell membrane to bind with the cannabinoid  
89 receptors present on the pre-synaptic membrane, and then carry them back to the post-synaptic  
90 neuron for degradation by their respective enzymes, FAAH or MAGL.

91 Endocannabinoids act on the presynaptic endocannabinoid receptors following their release from  
92 the post-synaptic neuronal membrane. The eCBs modulate neurotransmitter release by inhibiting  
93 the influx of intracellular calcium which in turn inhibits the release of neurotransmitters. eCBs  
94 undergo rapid re-uptake by the cells and are then degraded. Their half-life is quite brief. Following  
95 release, they undergo rapid reuptake by the cell and are then degraded. The production of eCBs can  
96 be stimulated by injury or excessive neuronal discharges [8]. ECS signaling comes in two  
97 forms—tonic and phasic. Tonic signaling establishes eCB tone or a basal level of signaling, while  
98 phasic signaling involves changes over time of eCB levels.

99 Omega-3 fatty acids are essential for the proper regulation of the ECS tone, since polyunsaturated  
100 fatty acids feed directly into the eCB signaling pathways [9]. These studies into the ECS were able  
101 to characterize the two cannabinoid receptors, the proteins that modulate their function and the eCB

102 family of compounds that encompass the ECS system. The ECS plays a major role in the regulation  
103 of many aspects of animal physiology. Today we know that the CB1 cannabinoid receptor is the  
104 most abundant GPCR in the human brain but is also present in many other organs such as the heart,  
105 blood vessels, liver, lungs, and the digestive system, as well as fat and sperm cells [10].

106 The CB1 receptor belongs to the Class A rhodopsin-like family of GPCRs. It is found primarily in the  
107 central nervous system with concentrations found in the cortex, hippocampus, outflow of the basal  
108 ganglia, and cerebellum. There can be both intra- and interspecies differences in the anatomical  
109 location of cannabinoid receptors in the ECS. It's important to note that CB1 in humans is not  
110 prevalent in the brain stem or medulla oblongata, the organs responsible for controlling vital  
111 autonomic functions such as breathing and heartbeat. This is a strong contributing factor to the  
112 safety profile of cannabinoids in humans and the main reason that it is nearly impossible to overdose  
113 on THC [11].

114 For dogs this is not true. Government studies in the 1970's determined that dogs have a very high  
115 density of cannabinoid receptors in the cerebellum and brain stem and medulla oblongata [12]. This  
116 concentration of CB receptors in the cerebellum explains "Static Ataxia", the neurologic reaction to  
117 THC that is specific to dogs naïve to THC. Static ataxia was first described in the literature by Dixon  
118 in 1899 [13]. Cannabinoid receptors are found to a lesser extent in the periphery of cardiovascular,  
119 immune, gastrointestinal, and reproductive tissues. CB2 receptors are located primarily in immune  
120 cells, among them leukocytes and those of the spleen and tonsils [14]. The CB1 and CB2 receptors  
121 share a significant degree of homology despite being located primarily in the CNS and immune  
122 system, respectively.

123 One of the functions of cannabinoid receptors in the immune system is the modulation of cytokine  
124 release. Activation of B- and T- cell CB2 receptors by cannabinoids leads to inhibition of adenylyl  
125 cyclase in these cells and to a reduced response to immune challenge [15]. Both CB1 and CB2 are  
126 cause a decrease in adenylyl cyclase activity and the cAMP pathway. They also stimulate  
127 mitogen-activated protein kinase (MAPK) cascades, modulate ion channels, and modify intracellular

128 calcium levels and subsequent neurotransmitter release [16-19]. Potassium channel activation can  
129 also serve as a signaling mechanism for the CB2 receptor [20,21].

130 Cannabinoid action is not limited to signaling outside of the cell. Fatty acid-binding proteins (FABP)  
131 are essential for the transport of cannabinoids into the cell. Once inside the cell they bind to  
132 cannabinoid receptors on the outer mitochondrial membrane and have several pathways: 1. Recruit  
133 nuclear transcription factors which modify gene expression [22] and 2. Regulate neuronal energy  
134 metabolism [23]. CRIP1a, another cannabinoid signaling protein, inhibits constitutive eCB signaling  
135 [24]. Mitochondrial CB1 receptors modify cellular respiration through inhibition of soluble adenylyl  
136 cyclase and reducing activity in the electron transport chain. Mitochondrial receptors may also play  
137 a role in the pro-apoptotic mechanisms of cannabinoids upon cancer cells, via the release of  
138 ceramide and its role in creating ER stress, leading to autophagy and if the cell cannot correct itself,  
139 apoptosis ensues [25].

### 140 **3. Veterinary ECS: Our Current State of Knowledge**

141 Most of what we know about the medical and health benefits of cannabis relates to humans and not  
142 animals. Many of the biological interactions of the endocannabinoid system occur across most of the  
143 animal species. Therefore, describing the medical and health benefits of cannabis as relates to  
144 humans, are starting points for evaluating cannabis as a therapeutic agent in veterinary species.

145 The CB1 receptor is highly conserved across all mammalian species, but there are significant  
146 primary sequence differences that have been discovered between the human and rat cannabinoid  
147 CB2 receptors and the newly cloned canine cannabinoid receptor, CB2. It was found that the binding  
148 affinities for canine CB2 receptor were 30 times less than those measured for human and rat CB2  
149 receptors. The functional properties of the cannabinoid CB2 receptor are highly dependent upon the  
150 level of receptor expression and the nature of the selected signaling pathway [26].

#### 151 **3.1 Anatomical Localization of Cannabinoid Receptors in the Dog**

### 152 3.1.1 Cannabinoid Receptor 1

153 Immunohistochemistry was used to anatomically localize the CB1 receptor in the normal canine  
154 nervous system. Nervous systems from a healthy 4-week-old puppy, three 6-month-old dogs, and  
155 one 10-year-old dog were evaluated. Strong “dot-like immunoreactivity” was found in the  
156 neutrophils of the cerebral cortex, cornu ammonis (CA), and dentate gyrus of the hippocampus,  
157 midbrain, cerebellum, medulla oblongata, and gray matter of the spinal cord. Dense CB1 expression  
158 was found in fibers of the globus pallidus and substantia nigra surrounding immunonegative  
159 neurons.

160 Astrocytes were consistently positive in all examined regions. In the PNS, CB1  
161 immunohistochemistry stained neurons and satellite cells of the dorsal root ganglia and myelinating  
162 Schwann cells in the PNS.

163 The younger dog in this study had lower CB1 expression in the brain. The density of receptor  
164 expression in human fetal and neonatal brain tissue was greater when compared to the younger dog  
165 examined. Lower CB1 expression has been found in aged rats in specific regions, most prominently  
166 in the cerebellum, cerebral cortex, and basal ganglia and less prominently in the hippocampus. This  
167 reduction in CB1 density with age in these rats was consistent with the findings in the older dog  
168 examined in this study [27]. Previous studies have identified CB1 receptors in salivary glands [28],  
169 hair follicles [29], skin, and hippocampus in dogs [30].

170 Immunohistochemistry was used to study the localization of CB1 receptors on developing canine  
171 embryo (30 days old) with a commercially available antibody. CB1 receptor immunoreactivity was  
172 found primarily in epithelial tissues and included most structures of the central and peripheral  
173 nervous system, inner ear, olfactory epithelium and related structures, eye, and thyroid gland [30].

### 174 3.1.1.2 Canine CB<sub>1</sub> Receptor Localization

175

- 176 • Cytoplasm of basal and suprabasal layer cells
- 177 • Hair follicle inner epithelial root sheaths and arrector pili muscles
- 178 • Undifferentiated sebocytes at the periphery of sebaceous glands
- 179 • Mast cells and fibroblasts
- 180 • Upregulated in atopic dermatitis

181

### 182 3.1.2 Cannabinoid Receptor 2


183

184 Clinically normal dogs have a homogeneous distribution of CB1 and CB2 receptors in all epidermal  
185 layers. This is different than in human epidermis where the CB1 receptor is mainly detected in  
186 epidermal spinosum and granulosum layers CB2 is detected mainly in basal keratinocytes. Both CB1  
187 and CB2 receptors have been found in the skin of healthy dogs and dogs with atopic dermatitis. The  
188 epidermis of dogs is thinner than that of humans (2–3 nucleated layers in the dog versus 6–7 in the  
189 human), which might account for this difference. Hyperplastic epidermal changes were observed in  
190 dogs with atopic dermatitis. Strong CB1 and CB2 immunoreactivity was identified in suprabasal  
191 keratinocytes. Weak CB1 and strong CB2 immunoreactivity was found in basal keratinocytes  
192 indicating upregulation of these receptors during inflammation. CB1 and CB2 agonists decrease  
193 mast cell degranulation. To summarize, cannabinoid receptor localization on the skin of the dog was  
194 found in the cytoplasm of epidermal and follicular keratinocytes, sweat and sebaceous gland  
195 epithelial cells, and the mesenchymal dermal cells [31].

196

#### 197 3.1.2.1 Canine CB2 Receptor Localization

198 

- 199 • Epidermis.
- 200 • Cytoplasm of cells in the basal and suprabasal layers.
- 201 • Hair follicles in the basal and suprabasal cells of the outer and inner epithelial root sheaths. 



- 202 • Mild immunoreactivity in cells of arrector pili muscles and secretory and ductal cells of
- 203 sweat glands.
- 204 • Sebaceous glands in the cytoplasm and peripheral reserve cells.
- 205 • Mast cells, fibroblasts, and endothelial cells.
- 206 • Lymph nodes.
- 207 • Strong B-cell zone immunoreactivity mainly in germinal centers of secondary follicles.
- 208 • Upregulated in atopic dermatitis.

209

### 210 3.2 Invertebrate ECS

211

212 The two cannabinoid receptors, CB1 and CB2, have been found in mammals, birds, reptiles, and fish.

213 In a study of seven representative species of invertebrates, McPartland used tritiated ligand binding

214 assays to characterize the cannabinoid receptors in *Ciona intestinalis* (Deuterostomia), *Lumbricus*

215 *terrestris* (Lophotrochozoa), *Peripatoides novae-zealandiae* (Onychophora), *Jasus edwardi* (Crustacea),

216 *Panagrellus redivivus* (Nematoda) [the beer mat nematode], *Actinothoe albocincta* [white striped

217 anemone] (Cnidaria), and *Tethya aurantium* (Porifera) [Orange Puffball sponge] [32].

218

219 Cannabinoid binding was detected in all species studied except for the sea anemone (*A. albocincta*)

220 and sponge (*T. aurantium*). The receptors were consistent with CB1 receptors but not CB2 receptors.

221 Three of the organisms tested, earthworm (*L. terrestris*), velvet worm (*P. novae-zealandiae*), and mat

222 nematode (*P. redivivus*), were compared to a standard CB1 ortholog in rat cerebellar tissue. A high

223 affinity binding interaction was observed at various concentrations characteristic of CB1 receptors.

224

225 The authors of this study hypothesize that cannabinoid receptors evolved in the last common

226 ancestor of bilaterians, with secondary loss in insects and other clades. After conducting a systematic

227 literature review, the authors found that cannabinoid receptors have been identified in sea urchins,

228 leeches, earthworms, hydra, lobster (*H. americanus* and *J. edwardi*), and the beer mat nematode (*P.*  
229 *redivivus*), but not the nematode (*C. elegans*). No binding was observed in sponges (Porifera).

230

231 In a separate study, McPartland found that insects (*Apis mellifera* [western honey bee]), *Drosophila*  
232 *melanogaster* [common fruit fly], *Gerris marginatus* [water strider], *Spodoptera frugiperda* [fall  
233 armyworm moth larva], and *Zophobas atratus* [darkling beetle]) are devoid of cannabinoid receptors.  
234 This loss of CB receptors is unique to comparative neurobiology, in that no other known mammalian  
235 neuroreceptor has been found to be missing in insects (Ecdysozoa). The authors suggest that the lack  
236 of cannabinoid receptors in insects is due to their lack of ligands, in that insects produce little or no  
237 arachidonic acid, the precursor to the biosynthesis of endocannabinoids [33].

238

### 239 **3.3 The Endocannabinoid System and Disease** [11]

240

241 Most of the work that has been done to delineate the effect of the endocannabinoid system on  
242 various diseases, has focused on the human animal and experimental models with laboratory  
243 animals. The assumption can be made that comparable benefits can be achieved in veterinary species  
244 via comparable mechanisms of action. Clinical studies are needed in veterinary species to further  
245 delineate applications for phytocannabinoids and for endocannabinoid molecules.

246

#### 247 **3.3.1 Modulation of Anxiety and Stress**

248 Deficiencies in eCB signaling have been implicated in the etiology of a variety of conditions  
249 including PTSD, migraine, and fibromyalgia. Circulating levels of eCBs have been found to be  
250 markedly decreased in these disorders. The decline in circulating eCBs is correlated with  
251 anxiety-like behaviors. Chronic environmental stress leads to a down-regulation of CB<sub>1</sub> receptors  
252 combined with reduced levels of AEA but increased levels of 2-AG [34].

253 CBD in animal models was shown to have anxiolytic properties. Healthy human subjects who had to  
254 perform a stressful public speaking test (SPST), were given a 300 mg dose of CBD isolate which  
255 reduced their subjective anxiety comparable to anxiolytic benzodiazepam [35]. CBD has also been  
256 shown to ameliorate some of the undesirable effects of THC and when administered concurrently  
257 helps to temper the psychoactivity of THC.

258

### 259 3.3.2 Modulation of Inflammatory Conditions

260

261 Inflammation is a common condition that underlies the development and progression of many  
262 diseases and health conditions. The ECS has been shown both *in vivo* and *in vitro* to be involved in  
263 regulating the immune system through its immunomodulatory properties. Cannabinoids have been  
264 found to play a key role in several experimental models of autoimmune disorders such as multiple  
265 sclerosis, rheumatoid arthritis, colitis and hepatitis.

266

267 The cannabinoid receptor CB<sub>2</sub> is primarily found on the surface of immune cells. The primary  
268 function of these receptors is to modify the inflammatory response. These receptors protect the host  
269 from the pathogenesis of these conditions through the induction of multiple anti-inflammatory  
270 pathways such as suppression of T-cell-mediated immune responses. They induce apoptosis of  
271 T-cells and suppress pro-inflammatory cytokines and chemokines and also inhibit T-effector cell  
272 proliferation at the same times as stimulating proliferation of T-regulatory cells.

273

274 The cannabis plant contains many molecules that reduce inflammation. THC and CBD both have  
275 strong anti-inflammatory properties, while CBC, CBG, and THCV have also demonstrated  
276 anti-inflammatory properties. The apoptotic mechanism of phytocannabinoids upon immune cells is  
277 to activate of CD95 which induces both Bcl-2 and caspase cascades. Cannabinoids have also been  
278 demonstrated to promote the production of anti-inflammatory interleukins such as IL-10 while

279 inhibiting the production of pro-inflammatory cytokines such as TNF- $\alpha$  in a CB<sub>1</sub> - dependent fashion  
280 [36].

281

282 Non-steroidal anti-inflammatory drugs (NSAIDs) produce their anti-inflammatory response  
283 through interactions with the ECS. Acetaminophen is metabolized in the liver resulting in  
284 *N*-arachidonoylphenolamine (AM-404) which acts as both a cannabinoid receptor agonist and eCB  
285 reuptake inhibitor [37]. These interactions also block the conversion of arachidonic acid into  
286 inflammation and pain promoting prostaglandins [38].

287

288 Terpenes can also have anti-inflammatory activity. Among the terpenes,  $\alpha$ -pinene,  $\beta$ -myrcene,  
289  $\beta$ -caryophyllene bind to the prostaglandin receptors (PGE1 and/or PGE2) to produce an  
290 anti-inflammatory effect.  $\beta$ -caryophyllene is the only terpene known to bind to cannabinoid  
291 receptors thus attenuating inflammation in a CB<sub>2</sub> - receptor dependent fashion [39].

292

### 293 3.3.3 Modulation of Pain

294

295 Endocannabinoids modulate neural conduction of pain signals by both reducing the nociceptive  
296 neural signal of pain, and by reducing inflammation by means of activation of cannabinoid  
297 receptors, either by endogenous ligands or  $\Delta$ -9-THC.

298

299 CB<sub>1</sub> receptors modulate neurotransmitter release in the brain and spinal cord. CB<sub>1</sub> receptors are also  
300 present in nociceptive and non-nociceptive sensory neurons of the dorsal root ganglion and  
301 trigeminal ganglion, as well as macrophages mast cells and epidermal keratinocytes.

302

303 CB<sub>2</sub> receptors are found in cells of hematopoietic origin. There are few CB<sub>2</sub> receptors in the brain,  
304 spinal cord, and dorsal root ganglion. CB<sub>2</sub> receptors will up-regulate in response to peripheral nerve

305 damage. These cannabinoid receptors regulate neuroimmune interactions and interfere with  
306 inflammatory hyperalgesia.

307

308 The endocannabinoids anandamide and 2-AG are produced in tissue that has been injured, and  
309 activate cannabinoid receptors to suppress the sensitization of the nerve to nociceptive signals  
310 and/or to suppress inflammation. The anandamide modulates pain by: 1. Inhibiting nociceptive  
311 signals at the synapse by activating CB<sub>1</sub> receptors; 2. Transformed by COX-2 enzymes into  
312 pain-relieving molecules (prostaglandins). 3) Reduces inflammation through activating CB<sub>2</sub> and other  
313 receptors. 2-AG plays a role in the descending modulation of pain during acute stress. Both  
314 molecules are produced as the body's first response to tissue injury [40,41].

315

#### 316 **3.3.4 Metabolic Effects**

317 Satiety in part, is modulated through the hypothalamic pro-opiomelanocortin (POMC) neurons.  
318 Activation of the CB<sub>1</sub> receptor inhibits the POMC neurons and results in appetite increase. This  
319 reduction in satiety can be attributed to the inhibitory effects of cannabinoids on the release of the  
320 appetite suppressant  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH). There is an inverse correlation  
321 between levels of orexin-A and  $\alpha$ -MSH. Orexin A induces hyperphagia by increasing levels of 2-AG  
322 [42].

323 Cannabis users who have hyperphagia, also known as "the munchies", eat more calories due to this  
324 inhibitory effect of cannabinoids. Paradoxically, users of cannabis are slimmer than non-users of  
325 cannabis. The prevalence of obesity is lower in regular cannabis users compared to non-users, even  
326 after adjusting for important variables such as age, sex, and tobacco smoking status. [43]. Cannabis  
327 users display lower levels of fasting insulin and better insulin sensitivity than their non-using  
328 counterparts [44]. These are the metabolic effects of the endocannabinoid system, due in part to the  
329 presence of cannabinoid receptors on the mitochondria.

### 330 3.3.5 Cancer

331 The ECS plays a key role in modulating cell differentiation, cell proliferation, and cell death.

332 Additionally, cannabinoids such as THC and CBD stimulate appetite and reduce the emetic

333 responses seen in chemotherapy. These qualities make the ECS an attractive target for use in cancer

334 therapy. Cannabinoids can also down-regulate certain cancers through modulation of gene

335 expression.

336 In lung cancer the administration of CBD results in an upregulation of the expression of the

337 intracellular adhesion molecules (iCAM) which in turn prevent metastasis [45].

338 In gliomas the administration of CBD results in reduction in the expression of pro-angiogenic factors

339 in a dose-dependent fashion. THC, when administered in conjunction with CBD, has been found to

340 be synergistic to inhibit proliferation and survival of human glioblastoma cells [46].

341 The *in vitro* addition of CBD to breast cancer cells was found to down regulate the expression of ID-1,

342 a large contributor to metastasis of breast cancer cells. [47].

### 343 3.3.6 The Role of Antioxidants and Neuroprotection

344 Cannabinoids act as antioxidants and neuroprotectants. The US National Institute of Health (NIH)

345 holds a patent on these compounds for this purpose [48]. As antioxidants, cannabinoids can

346 neutralize reactive oxygen species. Cannabinoids inhibit voltage-gated calcium channels resulting in

347 the inhibition of the release of glutamate. This neurotransmitter stimulates neuronal depolarization.

348 Glutamate is released during periods of ischemia and other traumatic brain events. In excess,

349 glutamate itself is toxic and can lead to neuronal cell death through excitotoxic stress [49].

350 Compounds with antioxidant properties are often neuroprotective, for instance through reduction of

351 toxic reactive oxygen species (ROS) produced during ischemic metabolism. Both THC and CBD

352 have been shown to have antioxidant properties [50]. THC and CBD are both able to prevent

353 glutamate induced neurotoxicity. The neuroprotective effect of these compounds is independent of  
354 their CB receptor binding activity. THC and CBD reduce ROS in vitro, similar to known antioxidants  
355 such as ascorbate and butylated hydroxytoluene (BHT). CBD has been shown to protect against  
356 cerebral ischemic injury [51], and also attenuates Alzheimer's-related neuroinflammation in animal  
357 models [52].

358 Terpenes have strong antioxidant properties and can also serve as neuroprotectants.  $\beta$ -myrcene, a  
359 common terpene in high THC cannabis but not low THC cannabis (hemp), protected against  
360 oxidative stress and histological damage induced by ischemia-reperfusion in a mouse model of  
361 cerebral ischemia. Terpenes in cannabis with antioxidant properties include  $\beta$ -caryophyllene,  
362 limonene, and  $\beta$ -myrcene [53]. Nearly all of the cannabinoids have been found to have potent  
363 antioxidant properties. [54,55].

364 The ECS is involved in the development of many neurodegenerative conditions. Cannabinoids have  
365 been shown to have neuroprotective properties, possessing the ability to reduce neuroinflammation,  
366 and promote neurogenesis [56-58].

367 In Alzheimer's Disease (AD) cannabinoids are able to clear the toxic beta amyloid ( $A\beta$ ) plaques  
368 associated with this disease. AD has been found to be associated with a loss of the body's natural  
369 production of eCBs, which has been defined as a clinical endocannabinoid deficiency syndrome  
370 (CEDDS), described later in this article.

371 CBD has also been shown to reduce the expression of genes implicated in the phosphorylation of the  
372 tau protein the hyperphosphorylation of which leads to the formation of neurofibrillary tangles that  
373 further contributes to the progression of the disease [59]. Furthermore, cannabinoids have been  
374 demonstrated to enhance the clearance of  $A\beta$  from the brain as well as prevent the inflammatory  
375 cascade that is produced by the accumulation of these mis-folded proteins intracellularly [60,61].

376 **3.3.7 Cardiovascular Modulation by the ECS**

377 Cannabinoids modulate blood pressure and heart rate, either increasing or decreasing blood  
378 pressure and heart rate, depending upon local conditions [62-64].

379 CBD has a direct effects on arteries, causing vaso-dilation, and subsequent hypotension as a result.  
380 This is a mild hypotensive effect, but could be problematic in uncompensated cardiac disease. CBD  
381 has a protective effect against vascular damage due to hyperglycemia from Type 2 diabetes, diabetic  
382 angiopathies and also with systemic inflammatory processes. The antioxidant and  
383 anti-inflammatory effects of CBD mediate these cardiovascular effects [65,66].

384 CBD demonstrated anti-arrhythmic effects following coronary artery occlusion in rats. This study  
385 found that these anti-arrhythmic effects were mediated through non-receptor pathways that did not  
386 involve the CB<sub>1</sub> receptor.

387 CB<sub>1</sub> antagonists increased blood pressure and left ventricular contractile performance in a group of  
388 rats bred to have spontaneous hypertension. Reductions in blood pressure, cardiac contractility and  
389 vascular resistance were mediated via the inhibitory effects of CBD on the enzyme FAAH by  
390 increasing the serum half life of anandamide in normotensive rats. CB<sub>1</sub> antagonists inhibited these  
391 effects. CB<sub>1</sub> antagonists lowered blood pressure better in the hypertensive rats versus normotensive  
392 rats. This may be due to the upregulation of CB<sub>1</sub> receptors in heart and aortic endothelium in  
393 hypertensive rats, but not the normotensive cohort [67].

### 394 **3.3.8 Modulation of Pulmonary Function**

395 Inhaled and oral THC can create bronchodilation up to two hours following administration. CB<sub>1</sub>  
396 receptor activation inhibits cholinergic contraction, which allows it to inhibit bronchospasms. This  
397 may be why asthma sufferers find some comfort from cannabis use [68]. CBD decreased pulmonary  
398 inflammation and improved pulmonary function tests in murine models of inflammatory lung  
399 disease, as well as improving pulmonary function in a model of COPD [69]. THC resulted in a  
400 reduction of allergen-induced mucus production [70].



### 401 3.3.9 Clinical Endocannabinoid Deficiency Syndrome (CEDS)

402 Russo has postulated that several chronic conditions may be due to deficiencies in eCB signaling.  
403 PTSD, migraine, IBS, PTSD and fibromyalgia are of particular note in this Clinical Endocannabinoid  
404 Deficiency Syndrome [71,72]. There are known mutations in ECS genes that contribute to such  
405 deficiencies, which helps to explain the genetic component of these diseases. Other causes that can  
406 affect the constitutive tone, or systemic level of eCBs include certain pharmaceuticals and diseases  
407 that can deplete eCB levels or interfere with eCB production. Human patients with mutations in  
408 CNR1 and DAGLA genes show signs of CEDS [73]. Human IBS patients with mutations in the CNR1  
409 gene, were found to also have altered rates of colonic transit [74]. With PTSD, fear extinction is  
410 impaired in patients who were homozygous for a CNR1 mutation [75].

411 These disorders all have in common markedly decreased systemic levels of eCB. Circulating eCB  
412 deficiencies are also inversely correlated with anxiety-like behaviors. Environmental stressors  
413 when chronic will down-regulate CB<sub>1</sub> receptors and reduce levels of both AEA and 2-AG [76].

### 414 3.3.10 Problems associated with the Endocannabinoid System

415 The psychoactive effects of THC, which is a CBR agonist, are undesirable in veterinary species. Dogs  
416 in particular will suffer from Static Ataxia upon exposure to THC at doses > 0.5 mg/kg IV [13]. As a  
417 result, states with medical or adult use marijuana laws, have found an increase in Animal ER  
418 admissions for THC toxicosis [77]. A report of the summary of calls to the Pet Poison Hotline found a  
419 relative good safety profile for CBD, and THC when given in moderate amounts [78]. Oral tolerance  
420 to THC can be achieved in the dog following 7-10 days of a low, sub-psychotropic of 0.05-0.1 mg/kg  
421 BID PO. Concurrent use of CBD in equal or greater amounts than the THC will assist in this process  
422 [12]. ECS stimulation that is excessive and prolonged can create memory deficits in humans. Upon  
423 cessation of prolonged ECS stimulation withdrawal symptoms will develop.

### 424 3.4 The Safety of Cannabidiol in Dogs

425 Two university studies, published in 2018 found CBD, when present in a full-spectrum hemp oil  
426 extract, to be safe when given at a high dose of 10 mg/kg/day for 6 weeks to Beagles, with only a  
427 mild and transient elevation in serum alkaline phosphatase, diarrhea, and no interference with  
428 anti-epileptic drugs. As CBD is metabolized via the P450 enzyme system, there is concern that the  
429 concurrent use of CBD with drugs that are also metabolized through that pathway may have their  
430 pharmacokinetics altered, which could alter their therapeutic value for a given patient [79].

431 Efficacy studies were performed for osteoarthritis at a dose of 2.0 mg/kg BID and clinical benefits  
432 were seen in the 16 client owned dogs with osteoarthritis who were recruited for this study [80].

433 Lower doses of 0.5 mg/kg BID have anecdotally been found to be effective for most dogs with  
434 osteoarthritis. The second efficacy study evaluated the ability of cannabidiol to reduce the number of  
435 breakthrough seizures in dogs with refractory epilepsy. At 2.5 mg/kg BID, the study found only a  
436 partial success for that pilot work. A larger study, funded by the AKC Canine Health Foundation is  
437 now being conducted using 4.5 mg/kg BID as the test dosage to evaluate whether it will be better  
438 able to extinguish these breakthrough seizures in these dogs [81].

#### 439 **4.0 Discussion**

440

441 From the research presented in this paper, its obvious that the endocannabinoid system in not just  
442 present in nearly all animals, but plays an integral role in maintaining homeostasis for a number of  
443 organ systems. The endocannabinoid system modulates the nervous and immune systems and other  
444 organ systems through a complex system of receptors and chemical signaling molecules to relieve  
445 pain and inflammation, modulate metabolism and neurologic function, promote healthy digestive  
446 processes, support reproductive function and embryologic development.

447

448 The future looks bright as cannabinoid research, in the post-cannabis prohibition era, is finally able  
449 to search for additional discoveries regarding the role the endocannabinoid system plays in the  
450 pathogenesis of disease, and the maintenance of health.

451

452 **5.0 REFERENCES**

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651

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