



Cannabis and cannabinoids

Mechanism of action and potential
as adjuvant supplements for pets

Veterinary technical journal for professionals

Historical context

Cannabis is a plant that has been cultivated and used for many years and with different purposes: the production of fabric, ropes or oil, as a psychoactive drug in religious ceremonies... Significantly, its use as a medicinal plant has been documented in civilizations as ancient as the Egyptian, where it was used to treat eye disorders or inflammations, or the Arabic, where its anthelmintic effect stood out. For its part, in the Chinese culture there are references to cannabis as a neuroprotective and analgesic agent ¹.

			Sumerian: A.ZAL.LA
			Akkadian: <i>azallû</i>
			Hieroglyphic: <i>shemshemet</i>
			Chinese kanji: <i>ma</i>
			Sanskrit: <i>bhāng</i>
			Persian: <i>shadanaj</i>
			Hebrew: <i>kaneh bosesem</i>
			Greek: <i>cannabis</i>

Figure 1: Cannabis in several ancient languages. Adapted from Russo et al. 2007¹.

If we focus on Europe, cannabis was used mainly at an industrial level to obtain fibers. It was not until the 19th century that it was introduced in the western medicine, becoming, by the end of that century, the third most used drug worldwide ². The increase in the recreational use of cannabis caused its illegalization, and therefore the research regarding its therapeutic applications dropped. The clinical interest reappeared in the 1960s, when its active molecules and its mechanism of action in the body were described. Since then, the number of research projects has never stopped growing, revealing an excellent therapeutic profile of cannabis, with great results in complex, chronic and even difficult-to-cure diseases.

What is cannabis?

There are different plants included under the name 'cannabis', 'hemp' or 'marihuana', all of them pertaining to genus *Cannabis*. They differ with respect to the amount of fiber they contain, the maximum size they reach, the active substances profile...

More than 421 molecules have been described in cannabis extracts, including terpenes, flavonoids, antioxidants, omega-3 and -6 fatty acids, proteins, minerals and B vitamins ³. Among them there is a group of chemical substances that stands out due to their singularity: **phytocannabinoids**. Cannabis contains more than 60 of these molecules, with two of them being the ones with the highest concentration: Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD) ⁴.

THC is a psychoactive molecule deemed responsible for the classic side effects of cannabis: increased appetite, euphoria, a dry mouth sensation, etc. Although THC has also proven a great therapeutic potential in several diseases (such as cancer ⁵ or IBD ⁶), being the molecule that makes the recreational use of marijuana possible, its presence in the cannabis plants has been limited through an European Union Regulation, so that only plants with a THC content not exceeding 0.2% may be used ⁷.

The second main molecule of the cannabis plant is CBD, that has sparked interest among the scientific community in the last years. One of the main advantages of CBD versus THC is that it does not produce side effects at a psychotropic level. Furthermore, it has become a more promising molecule at a therapeutic level due to its effects on the body: antioxidant, neuroprotective, anticonvulsant and analgesic, among others.

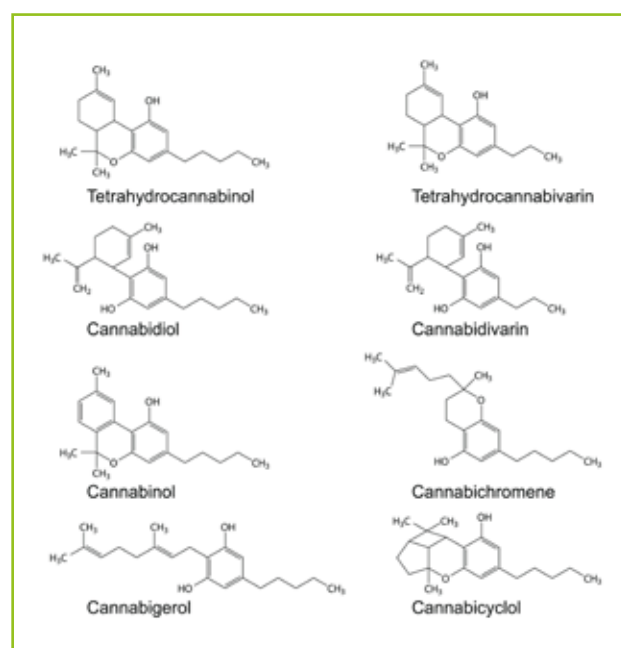


Figure 2: Main phytocannabinoids described in the cannabis plant.

We recommend two reviews that gather a great part of the technical articles that characterize the mechanism of action and the effects of CBD ^{3,8}. In this article we review the applications of cannabinoids, and especially of CBD, in different disorders at a human and veterinary clinical level.

How does cannabis behave in the body?

The mechanism of action of cannabinoids has been a scientific mystery until relatively few years ago. Raphael Mechoulam is the Israeli researcher that first isolated and synthesized THC. He discovered that this cannabinoid can bind to receptors in our body in a very specific way, producing an intracellular response. What sense did the presence of receptors for plant molecules make? The results indicated that these receptors could make a physiological sense in the body beyond cannabis. Indeed, later research described some endogenous molecules called endocannabinoids (endo = own, internal) ⁹.

We currently know many more details about the so-called **endocannabinoid system** (ECS), a complex regulation structure of mammals that includes these endocannabinoids, their receptors and the enzymes that regulate them ¹⁰. The ECS is present in all vertebrates and has been quite preserved throughout evolution ¹¹. Its main task is to ensure the balance between key physiological processes in the body, such as pain, memory, inflammation, temperature control, mood, or the liver and gastrointestinal function.



So far, six endogenous ligands or **endocannabinoids** have been described, among which anandamide and 2-arachidonoylglycerol (2-AG) stand out. These lipid molecules bind to the ECS receptors and activate an intracellular signalling pathway to trigger a physiological response in the body ¹².

The predominant receptors in the ECS are CBR1 and CBR2, although others such as TRPV1 have also been described ¹⁴. **CBR1** are mainly located in presynaptic neurons in the central and peripheral nervous system, where they develop a negative

feedback mechanism in the neuronal transmission that allow them to exert analgesic effects, a sense of well-being and others. Likewise, CBR1 have also been described in other tissues such as immune system, bone, skin and reproductive tissue cells ^{10,14,15}.

On the other hand, **CBR2** are found mainly outside the central nervous system: in immune system, hematopoietic, liver, bone, skin, etc. cells. Through the modulation of CBR2 in the immune system cells, the cannabinoids are able to exert an anti-inflammatory effect, regulating the release of cytokines and the migration of pro-inflammatory cells ^{10,14}.

An imbalance in the ECS can severely alter the processes that it regulates, such as the synaptic transmission, mood or the immunological balance... In fact, alterations in the ECS have been linked to different disorders, such as epilepsy, arthritis, atopic dermatitis, etc. Due to this, the molecules that can regulate the activity of the ECS, such as phytocannabinoids, have aroused a great therapeutic interest.

The Fundación Canna is a non-profit Spanish association that promotes the research and scientific dissemination of cannabis as a therapeutic tool. The essential concepts of cannabis as a therapeutic tool are very well reflected in one of their videos ¹⁶. Don't miss it!

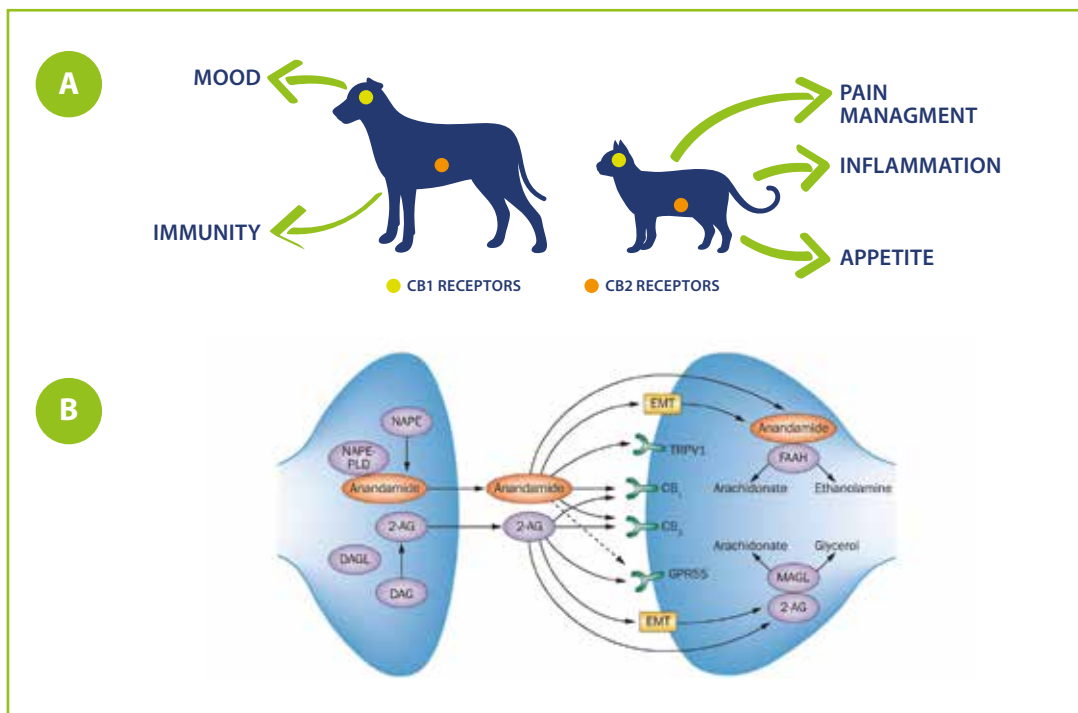


Figure 3: A) Main physiological processes in which the ECS participates. B) Basic functioning diagram of the endocannabinoid system at a synaptic level. Figure by Schico and Storr 2014 ¹³.

Cannabis for pets

The unique features of cannabis have raised a great interest within the medical community. Thanks to its ability to modulate the endocannabinoid system, that ensures the balance of key physiological processes, the products derived from hemp are used in the veterinary practice as a nutritional supplement in a great number of cases.

How do they work? Are they safe? When are they used? Here we will describe the key properties of the use of hemp in pets.

Biochemical profile

Hemp extraction oil is obtained from the aerial parts of the plant: flowers, stem, leaves and seeds. It mainly contains the phytocannabinoid **cannabidiol** (CBD), the most interesting molecule at a clinical level because of its anti-inflammatory, analgesic and anxiolytic effects^{3,8,14,17}.

The contents in THC in products for pets is usually very low (0.05%), and therefore no psychotropic side effects typically associated to cannabis consumptions will be seen. It has also been described that CBD blocks the effects of THC in the body. Therefore, the veterinary products usually have a very low THC content, and its possible residual effect is blocked by the high presence of CBD.

Regarding the clinical practice, it is more interesting to use products formulated with extraction oil obtained from **the whole plant**. This kind of ingredients include, besides CBD, much more phytocannabinoids (such as CBG or CBN), terpenes and flavonoids, among others. It has been shown that the different molecules help each other to exert a stronger effect than that of the molecules separately. This synergy is known as the *Entourage* effect.

On the other hand, there are other products formulated with powdered hemp seeds or hemp seed oil. They are regarded as highly nutritious due to their levels in omega-3 and -6 fatty acids, proteins and other vital substances. Nevertheless, in comparison with the whole plant extract, they contain very low CBD levels. Likewise, there are products formulated with pure CBD (*isolated* CBD) diluted in some kind of vegetable oil, whether olive or coconut oil. Although they may have an equivalent CBD content, they have a great disadvantage in comparison with the hemp extraction oils obtained from the whole plant: they do not include the rest of the molecules included in the plant (like the other phytocannabinoids, the terpenes or the flavonoids), and therefore the *Entourage* effect is lost and they will have more limited effects on the animal.

Therefore, in summary, **hemp extraction oil obtained from the whole plant shows the most complete and interesting biochemical profile at a veterinary level.**

Onset of action

Phytocannabinoids are lipophilic compounds that are better absorbed in the presence of oils or polar solvents, such as ethanol¹⁸. In comparison with an inhaled administration, the cannabinoids administered orally exert their effects later, but they last for longer.

A hemp extraction oil-based nutritional supplement will probably not show the speed of action shown by the common drugs, but it has a clear advantage over them: its much more limited side effects, especially when we talk about long-term treatments. According to previous experiences with the use of products with cannabis in pets, the length of time until their effects are visible is variable. In cases of arthritis, for instance, an improvement has already been seen in the first days after starting the treatment, or during the first month in cases of epilepsy, etc.

We must bear in mind that every pet possesses many variables that affect its response to cannabis: its general health status, the stage of development of the disease, the concomitant complications, its environment... Therefore, it is important to find the dose that can help the pet without causing it any harm.

Pharmacological safety

Cytochrome p450 is a group of enzymes that metabolises substances in the liver, several drugs among them. It has been seen that several phytocannabinoids, CBD among them, inhibit cytochrome p450, but very high doses are needed (10 mg CBD/kg) to inhibit the activity of cytochrome p450 *in vivo*^{19,20}. The products based on hemp extraction oil normally have much lower CBD levels. Along the same lines, in studies in humans, the inhibition of cytochrome p450 has never been seen with the doses used¹⁷. Anyhow, it is always recommendable that the veterinary staff supervises the combination of hemp-based products with drugs whose metabolism implies the participation of cytochrome p450.

On the other hand, cannabinoids have shown **anti-inflammatory, analgesic, anticonvulsant** and **antioxidant** properties, among others. When combined with other drugs with the same effect, it is possible to see an **additive or synergic effect**. Endocannabinoids and synthetic cannabinoids increased the effect of non-steroid anti-inflammatory drugs (NSAIDs),²¹ opioids²² or anticonvulsants in the case of epilepsy.²³ Because of that, in some cases it could be possible to reduce the amount of drug needed when administering a cannabis-containing supplement.

This is an important point, especially in the case of chronic patients, who are normally taking several drugs and, therefore, their kidneys and liver are under a great demand. In their case, the incorporation of a molecule such as CBD, with a good balance between efficacy and side effects, can open new and very interesting therapeutic paths.

Minimum age

Hemp extraction oils can be used at any age, always that the recommended guidelines for its administration are followed. In fact, one of the uses for this kind of products is in puppies/kittens, to alleviate the anxiety caused by the separation from their dam.

Along the same lines, several studies are currently being carried out on cannabinoids in newborn animals. As an example of this we have Dr. Martínez-Orgado, manager of the Neonatology Service at the Hospital Universitario Clínico San Carlos, in Madrid, that coordinates a research group in the study of the neuroprotective role of cannabinoids in a kind of neonatal lesion (hypoxia-ischemia). They have shown that CBD protects against the neuronal damage caused by the lack of oxygen in newborns.

Side effects

Nutritional supplements formulated with hemp extraction oil are normally very safe. Make sure that you obtain them from a reliable manufacturer, that carries out controls and tests in certified laboratories. The most problematic molecule is THC, that causes the side effects related to the consumption of cannabis. If the product has a very low concentration of this phytocannabinoid, it is expected not to see any psychotropic side effect associated to it. What is more, according to a study carried out in 2012, a minimum lethal dose of THC for dogs of 3 g/kg was established²⁷. This is, a dog should take more than 200 containers of a product with a 0.05% of THC.

At the same time, in the USA they have been working with hemp-based products for pets for years. In a field study of one of these products, the owners said that it was very useful in cases of pain, anxiety and sleep disorders²⁴. On the other hand, the most frequent undesirable effects were sedation and an increased appetite. Sedation is probably related to the anxiolytic effects of cannabis, that promote a state of emotional imbalance^{25,26}. Likewise, the increase in appetite can be a direct consequence of an increase in the pet's wellbeing and quality of life.

Bearing in mind the veterinary experience, **it is recommended to administer the products formulated with hemp extraction oil progressively**: starting with a small amount and increasing it gradually until finding a dose that can help each pet in each specific clinical situation. Because of that, in case that some of these undesirable effects are seen, the dose can be reduced to obtain a benefit without adverse reactions.

Scientific backup

If we search the term 'cannabis' in PubMed, we find more than 20,000 matches (September 2019). The prestigious journal Nature even dedicated a whole monograph in 2015²⁸.

There are different university research groups in Spain that are going deeper into the medical usefulness of cannabinoids, such as Manuel Guzmán, Professor of Biochemistry and Molecular Biology at the Complutense University of Madrid; Julián Romero, from the University Francisco of Vitoria; or Susana Mato, from the University of the Basque Country. Many of them are members of the Spanish Society for Cannabinoid Research²⁹.

At a veterinary level, three professionals in the USA that have been working with cannabis extracts for treating cats and dogs stand out: Gary Richter, Robert Silver and Caroline Coile. We can read about their clinical experiences and recommendations on the therapeutic use of cannabis on different publications^{2,30,31}.

There are also many clinical studies underway regarding the use of cannabis or CBD to treat different disorders: cancer, anxiety, epilepsy, etc. that can be checked at the *U.S. National Library of Medicine*³².

It is therefore undeniable that cannabinoids have fully entered the research field because of their **novel and safe mechanism of action**. They are also providing an answer to disorders that can currently only be tackled partially or symptomatically, and that is why the clinical expectations and interest are even greater

Effects of cannabis

Due to its **anxiolytic**, **neuroprotective** and **antioxidant** properties, cannabis-based products can be administered in:

- Anxiety, stress
- Separation from the dam
- Sleep and behavior disorders
- Neurodegeneration (e.g. cognitive dysfunction)
- Senior dogs and cats

Due to its **analgesic**, **anti-inflammatory** and **antioxidant** properties, they can be used as an adjuvant in cases of:

- Chronic pain
- Joint problems: arthritis, arthrosis...
- Autoimmune diseases
- Cancer
- Inflammatory diseases
- Stroke, epilepsy

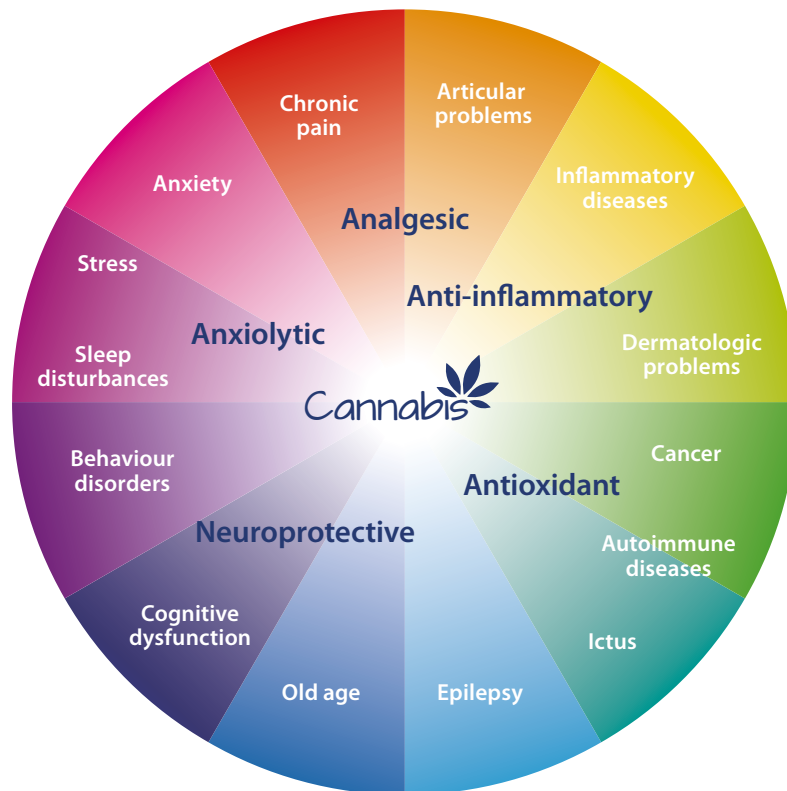


Figure 4: Effects and potential therapeutic applications of Cannabis in pets.

Therapeutical applications in the clinical practice

Arthritis

Pets may be affected by osteoarthritis (OA), an incurable degenerative disease that has become one of the main causes of chronic pain in dogs. Among the most frequent symptoms we have deformed joints, osteochondrosis and articular instability. Although it mainly affects senior pets, cases have also been described in young individuals.

Another kind of arthritis that can affect pets is immunological in nature, and it is known as rheumatoid arthritis. In these cases, the immune system leads a degeneration process of most of the limb joints. Its more frequent symptoms are general stiffness and a symmetrical joint involvement.

The ECS has been shown to be involved in the development of canine OA in a very recent study in which endogenous molecules that regulate the ECS activity were measured³⁴. Surprisingly, it was seen that the levels of two of them (2-AG and oleylethanolamide) were higher in the patients with OA in comparison with healthy dogs, this suggesting, for the first time, a direct involvement of the ECS in the development of this joint disease in dogs. The authors of the article point out that the CBR agonists, such as phytocannabinoids from *Cannabis sativa*, can become an innovative treatment for canine OA³⁴. In fact, therapies with different (synthetic or plant) cannabinoids have been already developed for human patients with OA or rheumatoid arthritis (RA)¹⁴. Moreover, in year 2000, researchers from the *Kennedy Institute of Rheumatology* in London showed that CBD has an anti-arthritic ability in mice³⁵.

In dogs suffering from OA, a nutritional supplement containing hemp oil obtained by extraction proved to reduce pain and increase the daily activity, thus improving comfort and quality of life^{35a}.

What is this anti-arthritic ability due to? It is due to the sum of the anti-inflammatory, immunomodulating, antioxidant and antinociceptive effects of cannabis extract. It is due to this that phytocannabinoids can be useful in the case of OA and rheumatoid arthritis.

Endocannabinoids help to preserve the integrity of cartilage through the inhibition of:

- The production of nitric oxide (NO)
- The activity of metalloproteinases
- The production of reactive oxygen species (ROS)
- The secretion of TNF- α and other pro-inflammatory cytokines¹⁴

Likewise, pre-clinical and clinical evidences support the therapeutic use of cannabinoids in cases of chronic joint pain.

Probably, **the decreased pain sensation** may be explained because of the **anti-inflammatory ability, together with the modulation of the neurological pain mechanisms**. The activation of the ECS has been shown to reduce the pain signals in the central and peripheral nervous system, probably through the modulation of the antinociceptive pathways and the inhibition of the inflammatory mediators responsible for pain (see the *Chronic pain* section). Along these lines, endocannabinoids and synthetic cannabinoids increased the antinociceptive effect of non-steroidal anti-inflammatory drugs (NSAIDs)²¹.

The endocannabinoid system appears as a great therapeutic target for the primary cause of osteoarthritis as well as to ease pain and inflammation.

Chronic pain

Chronic pain is defined as a disturbing and bothering sensation that persists for more than 3 months. It can be associated to concomitant diseases such as cancer, diabetes or arthritis, or it can be due to lesions that cause an inflammation of tissues, including the neuronal tissue. It does not have an obvious physiological sense, and in most of the cases, it is unresponsive to the usual treatment. It is deemed that between 10% and 25% of the human population suffers chronic pain, and this has already become a widely acknowledged problem. Although the incidence in dogs and cats is unknown, its importance in clinical practice is increasingly obvious³⁶. In a UK report, the veterinarians deemed osteoarthritis, dental and skin diseases, hearing disorders, vertebral and medullary pathologies and neoplasms as the most important causes of pain in dogs³⁷.

The physiological explanation of pain is the transmission of the potential danger in an area of the body to the nervous system. The peripheral nociceptors send this information through neurotransmitters, and one of the main ones implied in the signaling of pain is glutamate³⁸.

The ECS is one of the main pain control systems, and it works in parallel to the opioid system. It plays a key role in the development and the resolution of pain, as well as in the affective and cognitive aspects of pain³⁹.

Therefore, cannabinoids have proved their analgesic effects through different mechanisms³⁹. One of them is related to the neurotransmission of **glutamate** (Fig. 5). The activation of the glutamate receptor in the postsynaptic neuron induces the synthesis of 2-AG, that releases and activates CBR1 in the presynaptic neurons. This blocks the calcium inflow and stops the release of neurotransmitters. This is, cannabinoids exert a negative feedback mechanism (retrograde signaling), that can

act directly, by activating the CBR1, or indirectly, by modifying the activity of the enzymes that synthesize or break down cannabinoids (FAAH or MGL, for example).

Probably, the **antinociceptive effect** may be mediated by **the inhibition of the release of glutamate** in the areas related to pain²².

Another mechanism that has been described implies the stimulation of the glycine receptors, that modulate the response of the NMDA receptors. At the same time, through the modulation of CBR2, cannabinoids increase the release of endogenous opioids by the keratinocytes and immune system cells that, in turn, reduce the pain sensation¹².

Cannabis has also been used against the pain caused by chemotherapy. For example, **CBD reduced the neuropathic pain caused by paclitaxel**, without reducing the efficacy of chemotherapy. The modulation of the 5-HT1A receptor is, at least partially, responsible for this analgesic effect. Moreover, the cotreatment with paclitaxel and CBD showed additive or synergic effects in the reduction of the viability of the tumor cells⁴⁰.

Neuropathic pain is associated to the activation of microglia in the spinal cord and the brain, that induces a secretion of proinflammatory substances and reactive oxygen species. It is suspected that this increase in ROS is the initial cause of neuropathic pain. There are drugs containing phytocannabinoids that have proven their efficacy in the reduction of neuropathic pain⁴¹. Due to its immunomodulating effects, CBD can regulate the activity of microglia, while the tissue damage caused by it is reduced thanks to its anti-inflammatory and antioxidant effects³³.

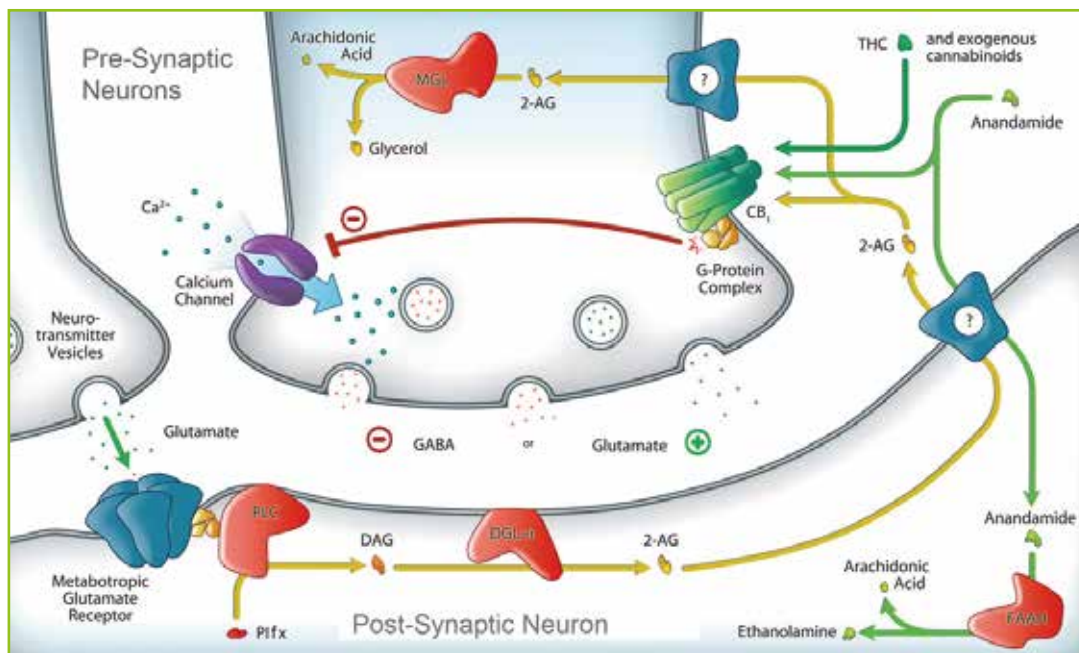


Figure 5: Diagram of the potential retrograde signalling system of the endocannabinoid system at the synaptic level. Figure adapted from Russo and Hohmann²².

The molecular evidences and the preclinical studies point towards a powerful **analgesic effect of cannabinoids**. Anyway, up to now, the results of clinical studies have not been very conclusive, probably due to the low number of participants, the short duration and the variety in the formulation of the cannabinoids, among other variables^{42,43}. Therefore, in the future it would be interesting to develop studies that include greater populations and administration control systems.

Even so, it seems to be more a clinical statistics than a therapeutic potential problem: the report drafted by the *National Academies of Sciences Engineering Medicine* affirms that **there are substantial or conclusive evidences that show the efficacy of cannabis in the treatment of chronic pain in adults**⁴⁴, highlighting, once more, the great potential of cannabis for treating pain.

Likewise, there are a pair of examples that serve, once more, as a great indication of this analgesic effect:

- **The improvement of pain was the most noted effect in the pets that were administered a cannabis-containing product**²⁵.
- In those countries where medicinal cannabis has been legalized, the use of opioids has dropped dramatically³⁶.

Skin problems

A reason that causes frequent visits to the veterinarian is **itching**, because it bothers both the pet and the owner. In these disorders there is a clear immune, nervous and inflammatory problem for which cannabis extract offers a therapeutic window.

The endocannabinoid system is implied in the maintenance of the proliferation, the differentiation and the survival of the epithelial cells, as well as in the tolerance of the skin immune cells. The imbalance between these processes can facilitate the development of disorders such as seborrhea, atopic dermatitis or psoriasis, among others⁵⁰.

Along these lines, a study discovered a overexpression of CBR1 and CBR2 in epithelia of **dogs with atopic dermatitis**⁵¹. These results open the door to the use of phytocannabinoids as a therapeutic tool for these disorders. An example of this is the administration of N-palmitoylethanolamide (PEA), a molecule that is considered a cannabimimetic: it does not bind directly to the CBRs, but it can regulate the endocannabinoid system indirectly. Surprisingly, a **study in Beagles** with allergic hypersensitivity showed that the administration of PEA has **anti-inflammatory, analgesic and antipruritic** effects⁵².

In addition, the immunomodulating ability of phytocannabinoids³³ can also help to control the skin disorders not only for reducing the annoying symptoms, but also to regulate the hyperactivity of the immune cells implied in their pathophysiology.

Epilepsy

Epilepsy is one of the most common chronic neurologic disorders in dogs, and it is characterized by an imbalance in the neuronal excitability that causes recurrent convulsions⁴⁸. It is deemed that this disease cannot be controlled in 25% of the dogs that suffer it⁴⁹, showing the urgent need for finding alternative treatments with less side effects.

The ECS is present in the central nervous system, modulating the neuronal electrical activity. Endocannabinoids are secreted by the postsynaptic neurons to activate a negative feedback mechanism, that generally reduces the synaptic transmission, and therefore they could be involved in the maintenance of the control of the convulsion threshold⁵⁰. It is therefore not surprising that several components of the ECS have been involved in neuronal pathologies. An example of this is a study in which alterations in the endocannabinoid levels in epileptic dogs were described⁵¹.

Phytocannabinoids, due to their ability to modulate the ECS activity, can be a logic option as a therapeutic tool against epilepsy, as evidenced by different studies in which the administration of CBD had **anticonvulsant, neuroprotective and antioxidant** effects in different in vivo epilepsy models^{3,8,17,33}. Another example: it was seen that CBD was able to block the toxicity of high levels of the excitatory neurotransmitter glutamate in an *in vitro* study⁵².

Although the exact molecular mechanism has not been described, the main studies indicate a critical role of the CBR1 found in glutamatergic neurons²³. When faced with an epileptic seizure, the body releases endocannabinoids, that bind to CBR1 and activate a cellular protection response through:

- The drop in the calcium inflow, that reduces the transmission by the glutamatergic neuron associated to epilepsy.
- The correction of the neuronal excitability through the modulation of the potassium channels.
- The reduction of the GABAergic function through retrograde signaling.

There is a great deal of research with regard to the use of cannabinoids against epilepsy in humans²³, including five Chinese studies³². Although the use of phytocannabinoids for canine and feline epilepsy has not been thoroughly studied, the great number of veterinary clinical experiences, coupled with the good results as anticonvulsants in human patients, suggest that phytocannabinoids can be an excellent therapeutic support tool for these disorders.

In fact, CBD has **additive or synergic effects with different antiepileptic drugs**²³.

Old age and cognitive dysfunction

As in humans, the different stages in the life of a dog or a cat imply changes in their cognitive functions and behavior. A certain deterioration of these parameters is deemed normal in old age. In the cases in which this degeneration is very fast, the cognitive dysfunction syndrome (CDS) may be developing. It is characterized by the clinical signs summarized with the acronym DISHA: disorientation, decrease in social interactions, alterations of the sleep-waking cycles, house soiling and lack of activity⁵³. Neuropathologically, mechanisms very similar to those seen in Alzheimer's disease have been described: loss of neurons and brain matter, amyloid pathology and reduced neurogenesis. The β -amyloid deposits are considered neurotoxic because they induce the synaptic degeneration, the depletion of neurotransmitters and neuronal death. Also, the oxidative damage also seems to be related to the development of the CDS.

In this sense, there are studies that have found a correlation between the severity of the CDS and the lipid and protein oxidation markers⁵⁴. On the other hand, the chronic hyperactivation of microglia is a common denominator in neurodegenerative disorders, such as Alzheimer's disease, CDS, Parkinson's disease or multiple sclerosis. These immune cells secrete proinflammatory cytokines and free radicals excessively, damaging the brain tissue.

Currently, the CDS is incurable. The current approach includes alleviating the damage caused by the excessive oxidative stress during ageing or supporting the mitochondrial function. Several nutritional interventions, such as the supplementation with antioxidants or omega-3 fatty acids

have shown to improve certain abilities of dogs with CDS⁵⁴. In this sense, the omega-3 fatty acids **protect and support the cognitive function** of pets.

Regarding cannabinoids, there are several studies that prove their ability to protect neurons against the lesions caused by inflammation or oxidative stress^{3,8,33}. There are different mechanisms that can explain this neuroprotection:

- Inhibition of proinflammatory molecules
- Antioxidant effect
- Blocking excitotoxicity
- Reduction of the calcium inflow

Cannabinoids have proven their ability in modulating the activity of microglia in several *in vitro* and *in vivo* models⁵⁵. In an Alzheimer's disease model in mice, the administration CBD reduced the expression of several proinflammatory cytokines released from microglia (such as iNOS and IL-1 β)⁵⁶, that caused an improvement in cognitive function⁵⁷. Likewise, in animals with memory loss, CBD helps to improve it⁵⁸. Interestingly, CBD also protects the neurons through the increase in the expression of antioxidant genes, such as superoxide dismutase⁸.

In summary, **cannabinoids, and especially CBD, exert protective effects on neurons under a high inflammatory or oxidative pressure, as in the case of the CDS**. The results of these *in vivo* studies are conclusive and, once more, show a great therapeutic potential of cannabinoids in neurodegenerative diseases.

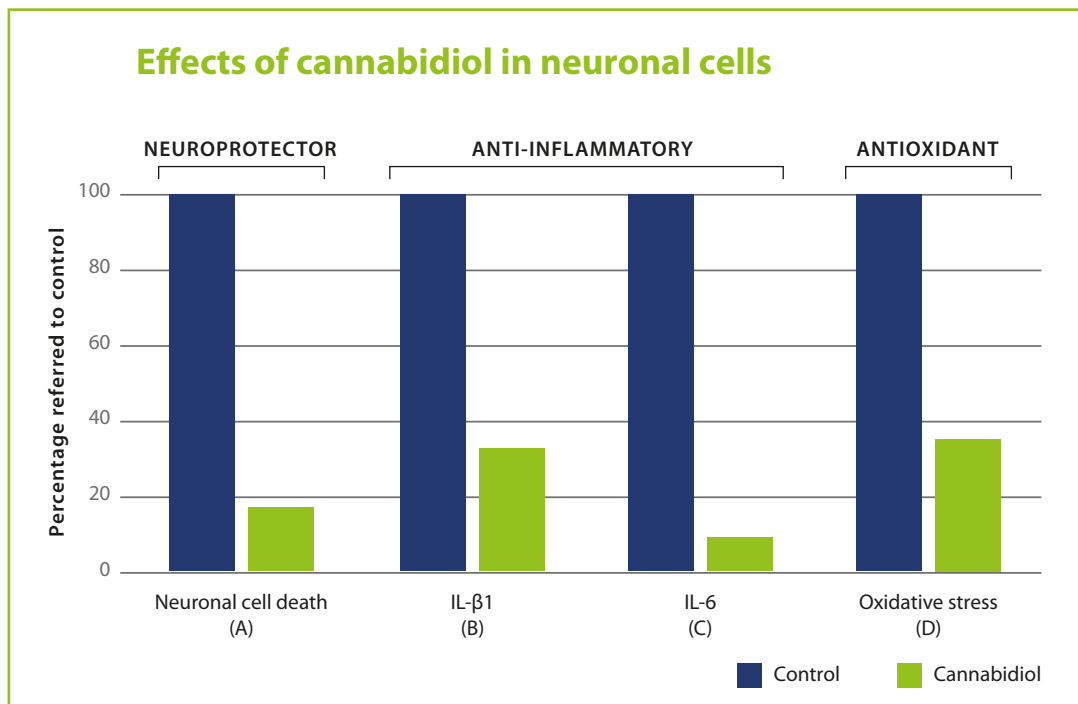


Figure 6: A) The treatment with CBD reduced the neuronal death in an Alzheimer cell model. B and C) The administration of CBD decreased the secretion of the pro-inflammatory cytokines IL- β 1 and IL-6 by microglial cells. D) CBD blocked the formation of β -amyloid-induced ROS in PC-12 cells. Adapted from (A) Scuderi⁵⁹, (B y C) Kozela⁶⁰ y (D) Iuvone⁶¹.

Anxiety and fear

Anxiety is the anticipation of future dangers with an unknown or imaginary origin that induce reactions in the body associated with fear, such as urination, breaking objects, excessive barking or whining... The causes that most frequently affect pets are separation from their owner, loud noises and the presence of new people or pets⁶².

There are several studies that link the ECS to the regulation of the disorders related to anxiety. A first evidence is the presence of the ECS in all the areas of the central nervous system implied with anxiety, fear and stress, including the hippocampus, the central and lateral amygdala and the prefrontal cortex⁶³. As we have previously seen, the ECS can regulate the synaptic transmissions through different mechanisms. As a matter of fact, the anxiolytic effect of exogenous cannabinoids has been linked to the modulation of CBR1 in presynaptic glutamatergic neurons. Along the same lines, the mice without CBR1 tend to have a more anxious behavior when faced with very aversive stimuli⁶³.

The activity of the ECS can also be modulated through its regulation enzymes. FAAH is the main enzyme that breaks anandamide down in the central nervous system. If its enzymatic activity is blocked with a drug or through genetic depletion, the average life of anandamide becomes longer and anxiolytic effects are observed⁶³.

Therefore, **it is not surprising that phytocannabinoids have an anxiolytic effect, because they can regulate the activity of the ECS at the central nervous system level and thus modulate the processes related to anxiety**²⁶.

The administration of phytocannabinoids has shown anxiolytic effects in a large number of *in vivo* studies, such as the Vogel conflict test or the elevated maze test^{3,27}. CBD, for example, reduced anxiety when compared to the placebo in human patients with social anxiety disorder. This anxiolytic effect coincided with a decrease in the limbic/paralimbic activity⁶⁴ and it was described that it is mediated by the interaction of CBD with the 5-HT1A receptor^{3,65}.

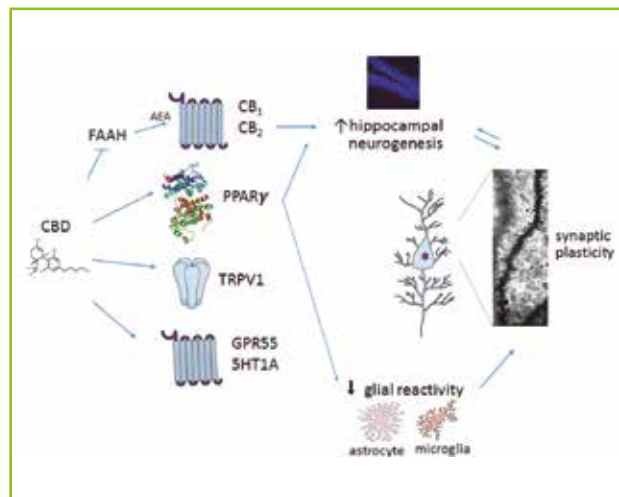


Figure 7: Mechanisms proposed to explain the effect of CBD in neuropsychiatric disorders. Adapted from Campos⁶⁶.

In fact, in a similar way to antidepressants or atypical antipsychotic drugs, it was seen that CBD induces plastic changes. For instance, the administration of CBD reduced neurogenesis in the hippocampus and the density of dendritic spines caused by chronic stress in a schizophrenia model⁶⁶.

In pets, a report revealed that **50% of the owners noticed an obvious improvement of anxiety in their dogs** when they administered them a **product containing cannabis**²⁵.

Therefore, in summary, the preclinical evidences show, conclusively, that phytocannabinoids such as CBD have an anxiolytic effect. Clinical research goes along the same lines and supports the data obtained, and therefore, once more, phytocannabinoids arise as a great therapeutic tool for controlling anxiety in different neuropsychiatric disorders.

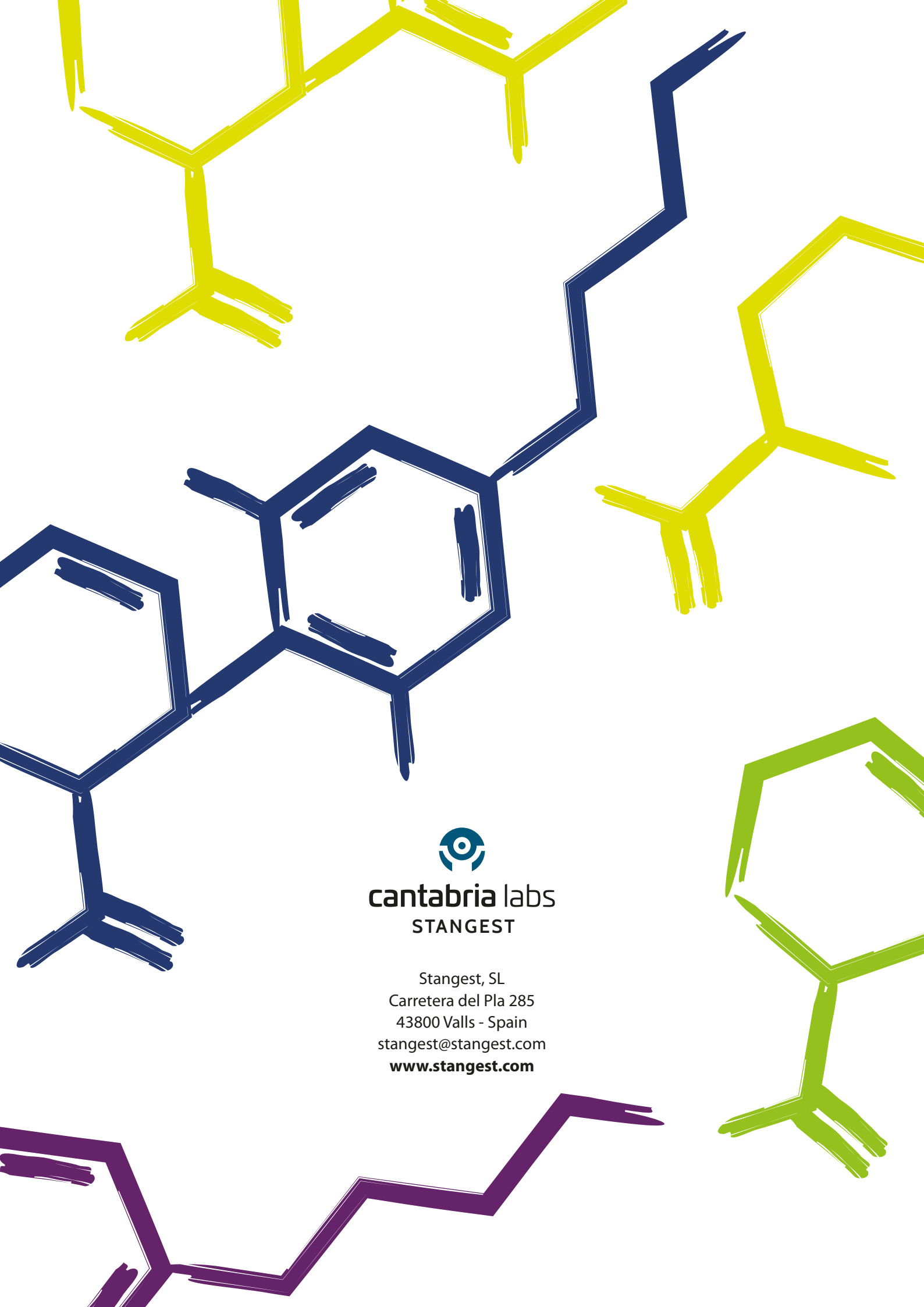
CONCLUSIONS

Cannabis went from being one of the most used plants in the 19 century to an almost non-existent use due its prohibition. Currently, many scientific studies are characterizing its activity and thus demonstrating its unique relationship with the organism. Thanks to its ability in the modulation of the endocannabinoid system, that ensures the balance between key physiological processes, a new research field has been opened not only regarding the functioning of the body, but also the unique therapeutic potential of cannabis.

There are many chronic pathologies that represent a clinical challenge due to its complex etiology and the difficulty to control their symptoms. In many occasions, the treatment with conventional medicines offers partial solutions or implies undesired side effects at a long term. Cannabis can be useful as an adjuvant in a wide range of these pathologies, becoming a great tool in the human and veterinary clinical practice.

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