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Key Aspects to Consider about Beneficial and Harmful Effects on the Central Nervous System by the Endocannabinoid Modulation Linked to New Cardiovascular Therapies

Martin Gimenez VM[1], Mocayar Maron FJ[2], Kassuha DE[1], Ferder L[3] and Manucha W[4,5]*

[1]*Research in Chemical Sciences, Catholic University of Cuyo, Argentina*

[2]*Department of Morphophysiology, National University of Cuyo, Argentina*

[3]*Department of Pediatrics, University of Miami, USA*

[4]*Department of Pathology, National Council of Scientific and Technological Research (IMBECU-CONICET), Argentina*

[5]*Department of Pathology, National University of Cuyo, Mendoza, Argentina*

Abstract

The endocannabinoid system is closely related to the central nervous system and exerts a promising therapeutic potential on it and other systems such as the cardiovascular, mainly through its neuromodulatory, neuroprotective and neuroinflammatory effects. For this reason, when designing new treatments for different relevant pathologies such as hypertension, it is necessary to take into account the side effects that such therapies can cause at the neurological level. Deeping knowledge of each cannabinoid and the molecular mechanisms that can lead to undesired results is necessary. The present review analyzes the psychoactive consequences of anandamide and other similar substances such as other endocannabinoids, phytocannabinoids and synthetic cannabinoids, to assess their little-explored therapeutic potential in clinical investigations. Finally, the major behavioral tests most used until the moment to predict possible changes in the conduct of animals treated with various substances of cannabinoid nature are summarized.

Keywords: Cannabinoids; Cardiovascular system; Central nervous system; Anandamide; Neuroinflammation; Behavioral tests

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Correspondence:
Walter Manucha, Department of Pathology, National University of Cuyo, Mendoza CP5500, Argentina, Tel: +54- 261-4135000; Fax: +54-0261-4287370; E-mail: wmanucha@yahoo.com.ar

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Introduction

The classical Endocannabinoid System (ECS) is formed by the endocannabinoid (eCB) signaling substances, Anandamide (AEA) and 2-Arachidonoyl Glycerol (2-AG) as well as their G-protein coupled cannabinoid receptors, CB1 and CB2. Lately, new receptors sensitive to Cannabinoids (CB) have been discovered, encompassing the orphan *GPR18* and *GPR55* receptors that are modulated by CB-like compounds and interrelate with ECS [1].

eCBs are signaling molecules of lipid nature implicated in processes such as appetite, memory, reward, mood, and neuroprotection. One study revealed that activities that are not running could raise eCB's plasma levels related to mood or appetite alterations. Augmentation of eCB's may be associated with the pleasurable and rewarding effects of exercise, singing and finally some of the long-range beneficial effects on memory, cognition and mental health [2]. Furthermore, the ECS has well-settled roles in neurogenesis, neuroinflammation and synaptic plasticity [3]. Relevant to it, several constituents of the ECS are located along the Central Nervous System (CNS) and participate in homeostatic functions of great importance such as cardiovascular regulation [4] (Figure 1).

The cardiovascular effects of cannabinoids are intricate, implicating the CNS, cardiac muscles, vasculature, and immune cells, the latter particularly in pathophysiological conditions. The Rostral Ventrolateral Medulla (RVLM), nucleus tractus solitaries, and periaqueductal gray are examples of CNS sites where CB1 receptors, the primary mediators of this type of response, are concentrated [5]. For example, stimulation of the CB1 receptor in the RVLM causes the growth of Renal Sympathetic Nerve Activity (RSNA) and Blood Pressure (BP) in rats [6]. Moreover, systemic

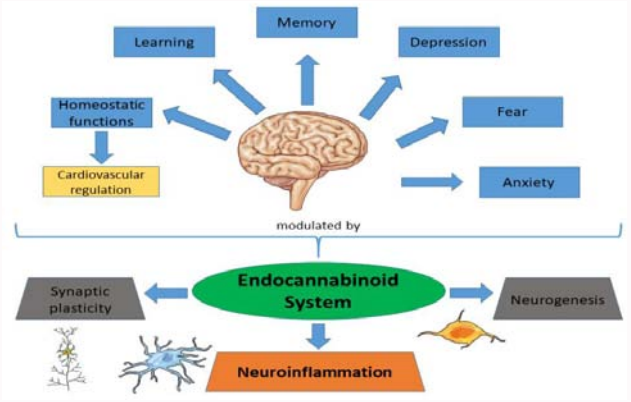

Figure 1: Endocannabinoids system in the neuroprotection-neuroinflammation cross-talk modulation.

administration of N-arachidonoyl glycine, a *GPR18* agonist, lowers BP by the activation of *GPR18* receptor localized in RVLM [7]. One of the most important cardiovascular effects of eCBs is the vasodilator, which, at the central nervous level, could be mediated by inhibiting the release of norepinephrine from perivascular sympathetic nerves through CB1 receptors [8,9]. The inhibition of the Fatty Acid Amide Hydrolase (FAAH), the primary enzyme liable for eCBs hydrolysis, also has cardioprotective effects on patients who suffer anxiety and other affective disorders where the levels of eCBs and CB1 receptors are altered. This nervous alteration increases the predisposition to suffer from various cardiovascular diseases. Thus, it has been described that FAAH inhibitors are capable not only of acting as anxiolytics and antidepressants but also have an antiarrhythmic response mediated by CB1 [10]. Moreover, CB2 receptors also play an essential role at the CNS and the cardiovascular level since they can modulate the neuroinflammation caused by different cardiovascular disorders such as ischemic brain injury in early stages, acting as immunosuppressant's [11].

Despite the beneficial effects of ECS regulation on the cardiovascular system, it is clear that this influence is also capable of compromising the CNS, which does not always produce the desired results. The most representative example of this is rimonabant, the first specific CB1 receptor antagonist used in the clinic for its capability to decrease food intake, enhance cardiometabolic parameters and stimulate weight loss. However, psychiatric adverse effects, related to an elevated risk of depression and suicidal thoughts, resulted in the withdrawal of this drug from the market. After this fact, clinical research with drugs that could affect the CNS has decreased markedly [12]. This conditioning supposes to waste new and necessaries potential therapies with cannabinoids that could be very effective for different types of diseases such as cardiovascular pathologies and others.

The present review proposes to analyze the psychoactive effects of Anandamide (AEA) and other related substances such as other endocannabinoids, phytocannabinoids and synthetic cannabinoids, to evaluate their weakly exploited therapeutic potential in clinical investigations. Additionally, the primary behavioral tests used to predict possible changes in the animals treated with different substances of cannabinoid nature are summarized.

Beneficial effects of ECS modulation on CNS

Concussive Traumatic Brain Injury (TBI) is the principal kind of brain injury in young adults as well as a risk factor for the appearance of chronic traumatic encephalopathy and other neurodegenerative pathologies in old age. TBI pathology usually remains refractory to presently available drugs [13]. Based on prior knowledge that genetic suppression and pharmacological FAAH blockade decrease anxiety and ameliorate memory, and emotional responses in humans like rodents, one study found that treatment with PF04457845, one novel FAAH inhibitor that selectively elevated the brain levels of AEA, improved locomotor function, memory, and learning in a mouse model of TB1 [14]. The accumulation of microglia, astrocytes, and the proinflammatory cytokines expression such as TNF-α, IL-6, and IL-1β in the ipsilateral TBI mouse cortex and hippocampus were considerably decreased by drug treatment. The enhanced locomotor function and working memory were partially regulated by CB1 and CB2 receptors activation, whereas the improvement in the spatial learning and memory seemed to be CB1 receptor-dependent. These results suggested that the FAAH selective inhibition has enormous potential in the therapy of TBI [13]. Moreover, using a mouse model of TBI, it has been discovered that the treatment of post-injury chronic with PF3845, another potent and selective inhibitor of FAAH, reversed the impairments in fine motor movement induced by TBI, working memory dependent on the hippocampus, and anxious type behavior. It decreased dentate gyrus neurodegeneration and up-regulated the Bcl-2 and Hsp70/72 expression in both hippocampus and cortex. PF3845 also blocked the elevated production of the amyloid precursor protein, reestablished the synaptophysin levels in the ipsilateral dentate gyrus, and prevented dendritic loss. Additionally, PF3845 repressed the inducible nitric oxide synthase and cyclooxygenase-2 expression and improved the post-TBI arginase-1 expression, indicating a change of macrophages/microglia from M1 to M2 anti-inflammatory phenotype [15]. So far, clinical data confined to safety studies with AEA hydrolysis inhibitors have shown that in the doses used, they have good tolerance and do not show signs of "cannabis-like" behaviors [16]. Furthermore, the endocannabinoid degradative enzymes manipulations, CB1, and CB2 receptors have shown promise in the modulation of cellular and molecular hallmarks of TBI pathology such as cell structure and remodeling, cell death, neuroinflammation, excitotoxicity and cerebrovascular breakdown [11]. Moreover, it is known that dual blockade of FAAH and TRPV1 interferes with contextual fear memory by promoting CB1 receptor activation induced by AEA in the dorsal hippocampus. This fact may conduct to novel pharmacological treatments for Post-Traumatic Stress Disorder (PTSD) [17].

Also, some researchers suggest the idea that eCBs participate in the cognitive processes regulation such as the spatial and emotional memories consolidation with an important influence of environmental variables, for example, the light-dark cycle [18]. Preliminary studies in humans have also suggested that the therapies with cannabinoids may reduce PTSD related symptoms, including nightmares [19].

Recently, AEA analogs have also been well identified for its potential neuroprotective effects in neutralizing the degeneration of Alzheimer's Disease (AD) brains. It was reported that dipotassium N-Stearoyl Tyrosinate (NSTK), a synthetic AEA analog, could increase spontaneous locomotor activity, low anxiety-like behavior, and improve the spatial memory shortfalls in a transgenic mouse model with Alzheimer [20].

The most abundant and important psychoactive compound in

marijuana, Δ (9)-tetrahydrocannabinol (Δ (9)-THC), has medicinal properties but also provokes undesirable detrimental consequences on cognitive function. Of particular interest, series of studies were performed in squirrel monkeys in order to contrast the impact of different kinds of cannabinergic drugs on several assessments of performance including cognitive flexibility, learning, short-term memory, attention, and motivation. Drugs studied included Δ (9)-THC, URB597, AEA, and its stable synthetic analog methanandamide. Altogether, these data suggest that AEA and its metabolically stable forms can have minor collateral effects on cognitive performance than Δ (9)-THC, probably giving a therapeutic benefit in clinical circumstances [21]. In this sense, the ECS manipulations could provoke therapeutic effects with a minor risk of unfavorable cannabis-like harmful effects [22]. Cannabis use has been related to a higher chance to develop schizophrenia as well as the worsening of their symptom. By the contrary, clinical studies have disclosed an opposite relationship between the cerebrospinal fluid AEA levels and symptom seriousness, indicating a therapeutic potential for drugs of endocannabinoid nature. Likewise, preclinical studies have demonstrated that these active pharmaceutical ingredients may reverse different behavioral problems in an animal model of schizophrenia. The subjacent mechanisms to the differences among the actions of exogenous and endogenous cannabinoids are, at present, undetermined [23]. Morena et al. [24] reported that aversive stimuli produced augmented concentrations of AEA in hippocampus, amygdala, and medial prefrontal cortex of rodents in a short time after training. Moreover, it has been suggested that this increment in cannabinoid levels controls emotional activation consequences on memory consolidation. Moreover, it was observed that the positive effects of exercise on spatial memory depend on the CB1 receptor signaling stimulation by increasing the concentrations of AEA [25]. Besides, other authors have reported that CB1 receptors activation mediated by AEA intensifies oscillatory activity and spontaneous bursting in the thalamus, suggesting that eCBs could have an essential role in control mechanisms of the sleep-wake cycle and activation level [26]. Also, it is important to mention that ECS regulates memory function in a differentiated way, depending on the stress level and excitation associated with the empirical context. Thus, ECS is an emotional regulator that cushions the stress and environmental context effects on cognitive processes [27].

Otherwise, methamphetamine toxicity is related to apoptosis and loss of dopaminergic neurons in the striatum analogous to what is found in various neurodegenerative pathologies. In this sense, one study assessed if ECS activation could decrease the neuronal toxicity provoked by large doses of methamphetamine on the dopamine system. The latest results indicated that ECS stimulation before the supply of an overdose of methamphetamine significantly reduced its neuronal toxicity by the activation of CB2 receptor and highlighted a protecting function for the ECS against toxic drug action and other external injuries to the brain [28]. Also, there is strong evidence on the protective role at the neuronal level of the eCB signaling cascade in several epilepsy models. In particular, augmented concentrations of eCBs prevent convulsions induced by Kainic Acid (KA). It was found that both AEA and its biosynthetic enzyme considerably rose in the hippocampus of younger rats treated with KA, while decreased in adult rats. The study indicated that the rise of endogenous AEA remarkably reversed epileptiform bursting induced by KA in rats [29].

To highlight, Human Immunodeficiency Virus type 1 (HIV-1) is considered a chronic pathology that mainly affects the brain and provokes HIV-1-associated neuronal and cognitive upsets. HIV-1 does not infect neuronal cells but generates viral toxins, such as Transactivator of transcription (Tat), that disturb the equilibrium of neuronal calcium resulting in synaptodendritic lesions and cell death. In particular, it was evaluated the neuroprotective effects of AEA and 2-AG on Tat excitotoxicity in primary cell cultures of Prefrontal Cortex Neurons (PFC), and if CB receptors are related with this neuroprotection. The treatment with AEA and 2-AG decreased excitotoxic concentrations of intracellular calcium and induced neuronal viability after Tat exposure, which was avoided by rimonabant, a CB1 antagonist, but not by AM630, a CB2 antagonist. In general, these results suggest that eCBs provides protection to PFC neurons from *in vitro* Tat excitotoxicity through a mechanism mediated by CB1 receptor. Therefore, the ECS also might have hopeful targets for the therapy of neurodegenerative disorders linked with HIV-1 infection (Figure 2) [30,31].

Adverse effects of cannabinoids and related substances

It is necessary to consider that CB1 and CB2 receptors have both likenesses and dissimilarities in their pharmacological behavior. Both admit several types of agonist and antagonist compounds and generate an array of different responses. Alternative splice variants, natural polymorphisms, distribution, phylogeny, and signaling pathways can also promote their pharmacological diversity [32]. For this reason, many times these same receptors that participate in all the beneficial effects of the cannabinoids mentioned in the previous section can also in certain circumstances, mediate different adverse effects caused by them. In this section, some of these situations are presented.

Thus, some studies suggest that high concentrations of AEA in the hippocampus and neocortex as a consequence of acute administration of URB597 -a FAAH inhibitor-, prejudice Long-Term Potentiation (LTP), learning and memory by the activation of CB1 receptor. These discoveries also indicate that pharmacological increase of AEA above normal levels is also harmful to the subjacent physiological responses [33]. Yu et al. [34] showed that AEA could disturb neuronal cell function both through an activator of the CB1 receptor, and by serving as a precursor of the arachidonic acid, a potent agonist of PPARβ/δ, a nuclear receptor in turn up-regulating multiple genes associated with cognition. Furthermore, it was displayed that FABP5, a fatty acid-binding protein, both stimulates the AEA hydrolysis into arachidonic acid and thus decreases brain eCB levels, and directly transports arachidonic acid to the cell nucleus where it gives it to PPARβ/δ, allowing its activation. In according, FABP5 removal in mice causes excessive accumulation of AEA, avoids PPARβ/δ activation in the brain, and notably affects learning and memory dependent on the hippocampus. Another study investigated if FAAH genetic deletion also impacts on age-related neuroinflammation. The results suggested that life-long increase of AEA levels alters microglial regulation and promotes pro-inflammatory modifications [35].

On the other hand, synthetic cannabinoids are a group of novel compounds which all act like THC but are chemically sundry. Anxiety, agitation, hypertension, paranoia, and seldom renal failure and myocardial infarction are some undesired effects of these compounds [36]. A case study reported that a potent synthetic cannabinoid (NM2201) produced several undesirable effects at CNS level such as agitation, double incontinence, lack of coordination on the left side, aphasia, generalized hypertonia, hyperreflexia, left

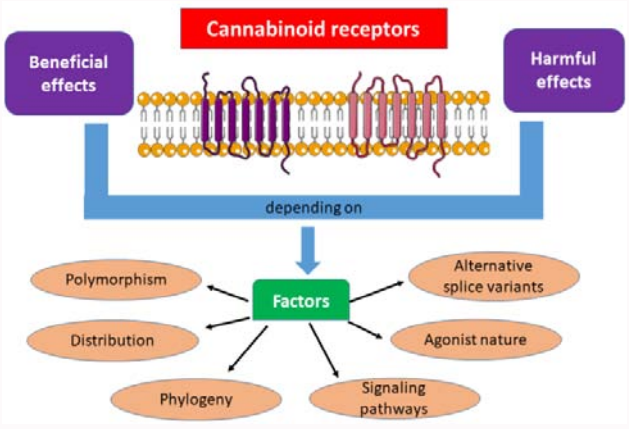

Figure 2: Endocannabinoids system modulation and its beneficial/harmful effects at central nervous system level.

hemiparesis, dense diaphoresis, fever, tachycardia, hypertension, and seizures [37]. Another synthetic cannabinoid, JWH-210, is a strong CB1 receptor agonist and also has adverse effects at the CNS level such as tachycardia, nausea, drowsiness, hypokalemia, hypertension, restlessness and agitation, diplopia, convulsions, syncope, cerebral convulsions and signs of sympathomimetic toxicity [38] (Figure 2).

ECS in the neuroprotection-neuroinflammation cross-talk modulation

CB receptors and associated orphan GPRs are highly expressed in the immune system and CNS and regulate many neurophysiological processes, including critical events linked to neuroinflammation. Indeed, these receptors have been suggested as novel therapeutic targets for many brain alterations in which neuroinflammation is a crucial characteristic, for example, Alzheimer's Disease (AD) and Multiple Sclerosis (MS) [1]. Aberrant activation of the Toll-Like Receptor (TLR)'s causes sustained neuroinflammation and has been related to psychiatric and neurodegenerative disorders. One study analyzed the effect of URB597 on neuroinflammation, physiological and behavioral troubles after TLR3 agonist administration to female rats. The results demonstrated that improving FAAH substrate levels inhibits the microglia/macrophage activation mediated by TLR3 and associated symptoms such as nociceptive responding, fever and anxiety-like behavior. These data provide evidence of FAAH could also be a new therapeutic target for neuroinflammatory diseases [39]. Moreover, mice with lack of FAAH showed reduced neuritic plaques, soluble amyloid levels, and gliosis. These data intensify the idea of a role for the ECS in neuroinflammation and open new approaches to the importance of regulating eCB levels in the inflamed brain. The same study informed that CB1 receptor blockade exacerbated inflammation in a transgenic mouse model of Alzheimer disease [40].

About the immunity of CNS, the activation of microglia is a polarized process spitted in potentially neurotoxic phenotype M1 and neuroprotective phenotype M2, preponderant during chronic neuroinflammation. In this context, the ECB system represents a promising target to control the equilibrium between both microglial phenotypes, since AEA as an immune regulator in the CNS. One study evaluated the effect of AEA on changes induced by lipopolysaccharide in rat primary microglial cultures. It was shown that the ECS plays a critical role in the modulation of neuroinflammation by control of the M1 phenotype activation. This effect was mainly mediated by the CB2 receptor, despite functional interaction with *GPR18*/*GPR55* may happen [41]. Mecha et al. [42] showed that 2-AG and AEA might be capable of acting by means of CB1 and CB2 receptors to stimulate the acquisition of an M2 phenotype in microglia cultures. It was presented three facts prove that the ECS is crucial for the acquirement of the M2 phenotype: (i) M2 polarization took place in the treatment with the two main endocannabinoids 2-AG, and AEA in cell cultures of microglia; (ii) CB receptor antagonists blocked M2 polarization; and (iii) M2 polarization was dampened in microglia from CB2 receptor knockout mice.

Also, it was demonstrated that AEA protects neuronal cells from oxidative injury in rodent models. It is known that neuronal NADPH oxidase 2 (Nox2), activation promotes to oxidative harm of the brain, and its inhibition can reduce this damage. In this regard, the mouse hippocampal neuron cell line HT22 was treated with hydrogen peroxide (H_2O_2) to imitate oxidative lesion of neurons. The protecting effect of AEA was evaluated by determining apoptosis, cell metabolic activity, cellular morphology, Lactate Dehydrogenase (LDH) release, antioxidant and oxidant levels and Nox2 expression and intracellular Reactive Oxygen Species (ROS). HT22 cells treated with H_2O_2 showed morphological modifications, reduced LDH release, lower metabolic activity, elevated concentrations of intracellular ROS and oxidized Glutathione (GSSG), decreased Superoxide Dismutase (SOD) levels, reduced Glutathione (GSH) and augmented NOX2 expression. AEA impeded these effects, a response suppressed by concurred administration of CB1 antagonist AM251 or CB1-siRNA. These results showed that Nox2 inactivation participates in cytoprotection induced by AEA against oxidative stress through CB1 activation in HT22 cells [43].

Additionally, a regulatory function of CB receptors and their agonists on the attenuation of the N-Methyl-D-Aspartate receptor (NMDAr) activation has been proved. Quinolinic acid (QUIN), an excitotoxic endogenous analog of glutamate, selectively stimulates NMDAr and has been shown to intervene in various neurodegenerative pathologies. WIN, a synthetic agonist of CB receptors and AEA, produced beneficial effects on the loss of cell viability induced by QUIN. The QUIN provoked lipid peroxidation, early mitochondrial dysfunction, and Reactive Oxygen Species (ROS) production, which was partially or wholly avoided by WIN pretreatment, but not when WIN was aggregated concomitantly with QUIN to rat brain synaptosomes cultures. These results suggested a modulatory and neuroprotective role of CB in the early toxic events provoked by inductor agents of this type of processes [44,45].

Other neuroprotective findings associated to ECS were recently established, and where the pretreatment with the Cannabidiol (CBD) phytocannabinoid in rats with right middle cerebral artery occlusion produced a considerable reduction in infarction, brain edema, and blood-brain barrier permeability in contrast to the group that received the vehicle. CBD also produced a down-regulation of TNFR1, TNF-α, and NF-κB expression. This fact demonstrated that CBD has a neuroprotective effect (mainly through deletion of TNFR1, TNF-α, and NF-κB) on ischemic injury [46].

From these findings, neuroscience as a branch of knowledge has made unremitting efforts to contribute to the development of multiple techniques and scientifically validated methods to properly study the effect of this type of substances. It is of central relevance to be able to apply reliable methodologies in the analysis of drugs that simultaneously involve systemic therapeutic effects and at the CNS level, which are not always beneficial for the latter (Figure 1).

Table 1: Main tests to evaluate effects observed in rodents as a consequence of the use of cannabinoids.

Behavioral Test	Substance	Observable Parameters	Effects
Elevated Plus Maze	Marijuana (blend of fitocannabinoids), marijuana and anandamide, WIN 55, cannabidiol, WIN 55, URB-597, AM-251, Tetrahydrocannabinol, and JWH133.	Duration and entries in the open and closed arms	No affect the cognitive performance, mild anxiolytic effect, biphasic effect on locomotors activity with marijuana, exploration and locomotion decrease with anandamide
			No impairment of spatial preference or memory, alterations in decision making
			None, anxiolytic effect, ansiogenic effect without previous stress, anxiolytic effect with previous stress
			CB receptors blocking provoke an anxiety behavior while activation exerts an anxiolytic action
			Males and females are equally susceptible to changes in emotional behavior when THC exposure occurs before the puberty stage; however, if THC exposure happens after puberty, males are more sensitive to the anxiolytic effects
			CB2KO (CB2 knock-out) mice are more anxious than WT (wild-type) counterparts
Novel Object Recognition	Marijuana (blend of fitocannabinoids), cannabidiol	Exploration period of unknown object and locomotion	No affect the cognitive performance, antidepressant effect, memory improvement
Open Field	Marijuana, WIN 55, marijuana and anandamide, cannabidiol, cannabidiolic acid, cannabidiol and anandamide	Periods in the peripheral and the central zone of the field	Mild anxiolytic effect , none, biphasic effect on locomotors activity with marijuana and exploration and locomotion dicrease with anandamide, no changes in general exploratory activity, anxiolytic effect, antidepressant effect
Somatic signs of withdrawal	Marijuana, tetrahydrocannabinol and JWH-018	Number of somatic signs	Dependence of marijuana
Tail suspensión	Cannabidiol, tetrahydrocannabinol and JWH-018	Immobility time, struggling time	Antidepressant effect, dependence of marijuana
Marble burying	Cannabidiol, tetrahydrocannabinol and JWH-018	Number of buried marbles	Anticompulsive effects, dependence of marijuana
Social interaction	JWH133, cannabidiol, tetrahydrocannabinol	Agressive behavior, time spent with the novel mouse	Reduction in the level of aggression by activation of CB2 receptor, memory improvement, low affection of social interaction
Prepulse inhibition	Tetrahydrocannabinol	Acoustic startle habituation	None
Forced swim	Tetrahydrocannabinol, cannabidiol and anandamide oleamide, JWH 133, AM251, and AM 630	Time of fight	Females are lesser sensitive to the antidepressant effects of THC, antidepressant effect, antidepressant effect of CB receptors agonists and attenuation of these effects by their antagonism
Conditioned place preference	WIN 55212-2	Time spend in an area associated with a drug	Higher propensity to develop addiction to cocaine
Forelimb grip strength	Cannabidiolic acid	Peak force	None
Light/dark box/ Fear conditioning-Passive avoidance	Cannabidiol, cannabidiolic acid	Freezing behavior	None, anxiolytic effect, none
Novelty suppressed feeding	Cannabidiolic acid	Feeding latency	None
Resident intruder	JWH133	Aggressive behavior	Reduction in the level of aggression by activation of CB2 receptor

Behavior tests used in the evaluation of cannabinoid effect

Concerning the above, it results in great relevance to know the effects on the CNS that can cause different treatments with cannabinoids and related substances. Multiple behavioral tests can be used in animals to evaluate the facts mentioned. The choice of one or another will depend on the pathology studied and the therapeutic use of the cannabinoid in question. Each test provides information on various aspects related to the different ways in which the behavior of a living being can be affected during these therapies. The most used behavioral tests so far in the field of research with cannabinoids and related substances are summarized below.

The Elevated Plus Maze (EPM) is at present one of the key study implemented in animals for evaluating anxiety produced by different cannabinoids. Anxiolytic or anxiogenic drugs increment or reduce, respectively, open arms exploration. Thigmotaxis, a tendency to be near to vertical surfaces, provokes those rodents to evade open arms in the EPM. This test consists in that rodents are placed in the center of the labyrinth, and a video-tracking system for 5 min records entries/duration in each arm. Other ethological parameters such as the time of permanence at the end of the open arms, considered the most anxiogenic area of this test, can also be observed. High activity in open arm (number of entries and duration of each one) reflects anxiolytic behavior. Some studies where it was investigated the effect of marijuana, AEA, THC, CBD and synthetic cannabinoids such as JWH133, WIN 55, 212-2, URB-597, and AM-251, showed, in general, an anxiolytic effect of the activation of CB receptors and an opposite answer by blocking it [47-56].

The Novel Object Recognition Test (NORT) is also a behavioral trial very common for the study of several aspects of memory and learning in mice and the influence of cannabinoids in them. The NORT is quite simple, and its duration is usually three days: habituation day, training day, and testing day. During training, the rodent explores two equal objects. Later, on test day, one of the training objects is substituted with a novel object. Due to the animal have an inborn preference for novelty it will spend most of its time at the novel

object, whenever the rodent identifies the familiar object. Also, the NORT can also be modified according to the requirements. The retention interval can be abridged to evaluate short-term memory or lengthened to assess long-term memory. Pharmacological treatment can be used at various times before training, after training, or before recall to analyze different stages of learning (i.e. acquisition, early or late consolidation, or recall). In general, the NORT is a relatively low-stress, effective test for memory in rodents, and is suitable for the finding of neuropsychological modifications following biological, pharmacological, or genetic manipulations. Some authors informed using this test, that marijuana does not cause cognitive changes in memory. Also, it has been shown that CBD causes antidepressants effects and improves memory [48,53,55].

The Open Field Test (OFT) is another of the most used experimental test to examine general locomotor activity levels, anxiety, and willingness to explore in animals (usually rodents) in the evaluation of cannabinoid effects on CNS. Rodents show a natural aversion to brightly lit open areas. Reduced levels of anxiety lead to augmented exploratory behavior. Incremented anxiety will result in less locomotion and preference to stay near to the walls of the field. The OF is an arena with barriers to prevent escape. Generally, the area is marked with a grid and square crossings. Behavioral patterns measured in the open field test include frequency of entrance to the central grid, time of permanence in the center or the periphery of the open field, standing, frequency of defecation and urination, among others. Some authors have reported that marijuana in the OFT produces a dual effect that consists of a first stage where the locomotor activity is increased and a later stage where an effect of reduced locomotion and exploration is manifested. It has also been observed that cannabidiolic acid causes a decrease locomotor and exploratory activity in OFT, while CBD and AEA provoke their increase, as a consequence of an anxiolytic effect of cannabidiolic acid and a mild antidepressant effect of CBD and AEA [47,49,53,57-59].

Somatic signs of withdrawal produced by different cannabinoids were measured in one study by putting each rodent into an empty, plastic test chamber within a sound-attenuating chamber equipped with a ventilator and white LED lighting. The dependent variables analyzed were incidences of paw tremors (clapping of the forepaws), and head twitches (twisting of the head). These dependent variables were selected because they have been observed systematically in mice submitted to THC withdrawal [60]. In another study where the same test was used to determine the signs of somatic abstinence, it was evaluated aspects such as body blemishes, tremors in the cheeks, blinking eyes, fluttering of the front legs, panting, genital licking, grooming, shaking of the head, ptosis, teeth blinking, contractions and yawns [47]. This test was used by some researchers to monitor drug dependence on substances with addictive effects such as marijuana and THC, its majority component.

Tail suspension test has been used for evaluating the action of THC and CBD on the behavior of mice that were hung by their tails with scotch tape from a horizontal bar situated roughly 40 cm above a counter and video recorded for 6 min. The time the mice actively fight was scored using the software. The active fight was considered as one or more legs recurrently kicking within one second or arching of the spine, without head movement. CBD has demonstrated antidepressant effects through this test, which has also been used in the evaluation of withdrawal syndrome caused by THC and synthetic cannabinoids [54,60].

The Marble burying test also analyzed the central nervous effects of THC and CBD. Plastic test chambers filled with wood bedding were putting within a sound-attenuating chamber equipped with a ventilator and LED lighting. Twenty-five clear glass marbles were laid through the top of the leveled bedding. Each animal was put singly into the chamber and enabled to explore for 20 min freely. At the end of the test, each rodent was removed, and the number of unburied marbles (≥ 1/3 of the surface showing) was registered then subtracted from the 25 total marbles. Locomotor activity was concomitantly recorded for the duration of the test by a camera placed on the top of the test chamber. The video data were examined in real time using the software. Dependent variables included: the number of marbles buried and the total time immobile. This test evaluates somatic and other behaviors including grooming, digging, rearing, scratching, and retropulsion. This test has confirmed the absence of compulsive effects after the administration of CBD and the dependence on marijuana observed after treatment with THC [58,60].

The social interaction test assesses sociability and social recognition memory by quantifying the amount of time an experimental rodent spends nearby a novel, caged "target" mouse, in comparison with an empty chamber and a chamber with a cage but no “target” mouse. In studies performed with THC and other CB2 agonists, each experimental animal was acclimated, first, in the empty middle chamber for 10 min, and then in the whole chamber for another 10 min. After this stage, a novel same-sex "target" rodent was placed in one of the cages in the lateral chamber, while the other wire cage was emptied. The plastic doors were unlocked for the 10 min test. The total time spent in each chamber and time spent immobile by mice were each measured using the software. In some studies, standard adversaries became temporarily anosmic by intranasal lavage with a 4% zinc sulfate solution one day before testing. This sort of rodent causes an attack response in its opponent but does not provoke the other animal or defend itself, since it is unable to perceive a urine pheromone of the experimental animals which functions as a signal for obtaining aggressive behavior in rodents with a normal olfactory sense [52,60]. Another variant of this trial, it is to use a second unknown standard adversary laid in the formerly empty chamber so that the test rodent had the option to explore either the known rodent (from the prior trial) or the new, strange rodent [48]. Analyzed parameters are usually social behaviors such as following (the pursuit of one animal by another), sniffing (mutual), climbing over and crawling under the partner and allogrooming, genital investigation (smelling the anogenital area); and non-social behaviors such as locomotion (horizontal exploration) and rearing (vertical exploration). This test has determined the reduction in the levels of aggression of rodents mediated by CB2 receptors, the improvement in memory after the use of CBD and the absence of effects that alter the social interaction in animals treated with THC [61].

Prepulse Inhibition (PPI) is a neurological response in which a fainter prestimulus (prepulse) overrides the response of an organism to an ensuing vigorous and alarming stimulus (pulse). The stimuli generally used are acoustic. If prepulse inhibition is high, the corresponding one-time startle response is low. A shortening of the amplitude of scare translates into the capability of the CNS to temporarily habituate to a strong sensory stimulus when a previous subtle signal is given to alert the organism. On the contrary, lackings of prepulse inhibition are evidenced in the disability to select out the needless information and they have been related with anomalies of the sensorimotor system. Such deficiencies are recognized in pathologies

like Alzheimer's disease and schizophrenia, and individuals under the effect of different drugs. For this reason, PPI has also been used for determining the cannabinoid influences on CNS. Some authors who have used this test have reported that THC has not caused any alteration in the habituation to acoustic stimuli [56,60].

Forced Swim Test (FST) is sufficiently sensitive to show augmented immobility in animals with depression. Rats are placed into a transparent Plexiglas cylinder loaded to 30 cm with water between 23°C and 25°C and coerced to swim for 5 min. The test is carried out in a small dark room and sessions were videotaped for posterior behavioral evaluation. Latency time of immobility and total time spent swimming/frightening and motionless are estimated as the dependent measures. Antidepressant drugs intensificate escapement behavior decreasing the immobility time values, while an increment in immobility is considered as a depressive-like behavior. The test has also been used in some studies to establish the potential depressive/antidepressive effect of cannabinoids. In general, the studies that have used this test, have led to conclude that the CB receptors activation by agonists such as THC, CBD, AEA, and synthetic cannabinoids, produces antidepressant effects that result in an increase in the time of fight of rodents in water [62,59,56].

Conditioned Place Preference (CPP) -a form of Pavlovian conditioning -is implemented to measure the motivational effects of different substances in rodents. The researchers can infer the animal's liking for the stimulus by mean of time that an animal spends in an area associated with a stimulus. It is possible to evaluate potential addictive effects using the rewarding effects of a drug as cannabinoids. One study carried out with the synthetic cannabinoid WIN 55212- 2 showed that the CB receptors activation by this agonist increases the predisposition to cocaine addiction, which is why its use is not recommended for recreational purposes [63].

The forelimb grip strength test is an extensively used method to appraise skeletal muscle performance in rodents. Animals are put with forelimbs grabbing a trapeze bar plugged into a digital force gauge, and then they are homogeneously pulled by the tail base away from a bar along the horizontal plane until grip was liberated and maximum force registered. One study where it was analyzed the effect of cannabidiolic acid on muscle strength of rodents showed that there is no effect associated with this substance that affects motor ability at the CNS level [57].

The Light/Dark Box (LDB) is a well-known animal model used in studies with cannabinoids to evaluate unconditioned anxiety responses in rodents. The apparatus consists of a closed, black acrylic chamber linked via a small gate to an open, white and illuminated acrylic chamber of the same proportions. Animals are put within the clear chamber in front of the entry hole and behavior is video registered for 5 min in order to evaluate the amount of entries within the light sector and their duration. Rodents typically spend the least time in the clear box than in the dark compartment. If rodents are injected with anxiolytic drugs, the percentage of time spent in the clear room will increase. Locomotion and standing, which is when the rodent stands up on its hind legs and is a sign of exploration, in the dark chamber also increase. When injected with anxiogenic drugs, animals spend more spent in the dark compartment. To highlight, only natural stressors such as light are used. Additionally, there is a variation of this method to evaluate aversive memory, and it consists in to apply a little electric footshock to the animal when it is in the dark box. This variation requires prior training and is known as Fear Conditioning test or also denominated Passive Avoidance test, which appraises hippocampal and amygdala dependent associative learning, therefore, a previous neutral stimulus provokes a dread reaction after it has been paired with an aversive stimulus. Based on this test, it was determined that CBD and cannabidiolic acid do not affect aversive memory in rodents. However, CBD could cause a mild anxiolytic effect in animals subjected to a previous aversive stimulus [48,50,57].

The Novelty-suppressed feeding is also used to check the antidepressant effect of cannabinoids and related substances. In this test is to measure the latency time from the onset of feeding and the amount of feed consumed when animals are placed in an illuminated box with a weighed quantity of standard laboratory chow. Antidepressive drugs produce stimulation of feeding in the rodents tested and vice-versa. The absence of central nervous effects of cannabidolic acid was demonstrated once again with this test since it did not alter the feeding behavior in the animals studied [57].

The Resident-intruder procedures test evaluates aggressive behavior in rodents. Resident mice are isolated for ten days before the experimental procedure. Later, intruder rodents of similar weight and age are placed in boxes in groups of five. Each session consists of putting an intruder animal in the resident's home box for 5 min. Animals receive two training sessions on the first day and two test sessions on the second day. The decrease in aggressive behavior mediated by CB2 receptors and induced by its synthetic agonist JWH133 was reconfirmed by using this test, with results similar to those observed with the social interaction test [52].

These tests, the main parameters that each one evaluates and the main effects that are observed with them as a consequence of the use of different cannabinoids in rodents are summarized in Table 1.

Conclusion and Prospects

The increasing study of the pharmacological properties of endocannabinoids and the modulation of ECS has revealed an exciting approach to the discovery and development of new drugs with multiple therapeutic properties. To highlight is especially interesting in the search for alternative treatments more effective than those used so far for diseases with high rates of morbidity and mortality worldwide such as cardiovascular diseases, particularly arterial hypertension. The simultaneous affectation of the CNS in these therapies is a fundamental factor to consider since many of them can interfere with cognition such as memory, learning and other aspects related to neuroinflammation (Figure 1). Despite this, it has been observed that endocannabinoids such as anandamide have few adverse effects at the CNS level, unlike the phytocannabinoids, synthetic cannabinoids and antagonists of CB receptors. In fact, these molecules act on common receptors due to a similar spatial configuration, however, their structure is not identical between them (Figure 2). For this reason, the competition and displacement of endogenous ligands of the ECS by exogenous cannabinoids provokes an alteration in the CNS normal functioning, leading to the associated side effects [64].

In this sense, the in-depth knowledge of each cannabinoid and the molecular mechanisms that can lead to undesired results is necessary. Researchers are the primary responsibilities that the use of cannabinoids in the biomedical field is no longer considered a taboo and conversely, begins to take advantage of its enormous therapeutic potential.

As future perspectives, new animal models could be investigated, and new behavioral tests developed to obtain increasingly precise and representative results of the effects of cannabinoids in humans. Additionally, new forms of drug administration could be used, such as nanostructured systems, which allow drug localization and targeting

to prevent the arrival of cannabinoids in the CNS and thus prevent the appearance of secondary psychoactive effects.

Author Contributions

All authors contributed to conception and design of the review, with substantial contribution to data acquisition, analysis and interpretation of the data, drafting of the article, and critical revision of the article for intellectual content.

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