Chapter 9

The Endocannabinoid System in the Vervet Monkey Retina

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Additional information is available at the end of the chapter

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Abstract

The main active compound found in the marijuana plant, tetrahydrocannabinol, is responsible for its psychotropic effects but also for its numerous beneficial actions such as appetite stimulation, nausea reduction, analgesia, and muscle spasm suppressor. Although cannabis consumption leads to some visual disturbances, the exact role of the endocannabinoid system (ECS) in normal vision is still unknown. Many studies have looked into the localization of this complex system (receptors, ligands, and enzymes) throughout the various components of the visual system of different animal models in order to obtain clues about its role. In fact, the retina, optic nerve, dorsal lateral geniculate nucleus, and visual cortices all express parts of the ECS. Manipulating this system pharmacologically or genetically has also an impact on visual function. In this book chapter, we provide the current understanding of how the ECS is involved in the functioning of the visual system and special emphasis is put on data obtained in monkeys, representing the most relevant animal model for visual neuroscience research. The mechanisms that control endocannabinoid (eCB) release and activation of cannabinoid receptors are discussed. We also propose a model highlighting the mechanisms involved in the regulation of photopic and scotopic vision taking advantage of the spatial specificity of the eCB signaling system and its physiological activation conditions.

Keywords: cannabinoids, retina, CB1 receptors, CB2 receptors, GPR55, vision, monkeys

1. Introduction

The medicinal use of cannabis can be traced back many thousands of years, but research on cannabinoids and the endocannabinoid system (ECS) was stimulated only in the mid-1960s

after the isolation of tetrahydrocannabinol (THC) from the marijuana plant *Cannabis sativa* [1]. Later on, in the 1970s, several research groups reported independently that this compound is mostly responsible for the therapeutic and psychotropic effects of cannabis. Until the late 1980s, it was thought that cannabinoids would act nonspecifically on membranes and channels, but the discovery of specific receptors that bind to cannabinoids (THC and cannabidiol specifically) changed the course of events. A first cannabinoid receptor, termed cannabinoid receptor type 1 (CB1R), was discovered and cloned [2, 3]. Since the endogenous receptors do not stand and wait for cannabis consumption in order to get activated, the existence of endogenous molecules that activate the cannabinoid receptors was suggested. Indeed, the discovery of endogenous molecules that activate these receptors was made shortly after. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG), the two most studied eCBs, then led to the discovery of specific enzymes that regulate the eCB levels. It is now clear that the ECS is present in many places in the organism and plays a neuromodulatory role at the cellular level. The eCB AEA and 2-AG bind to CB1R and CB2R with different affinities. There is also strong evidence that suggests that eCBs can target other receptors, particularly the putative "CB3" receptor GPR55 and the transient receptor potential vanilloid 1 (TRPV1) ion channel. Other eCB targets such as peroxisome proliferator-activated receptor (PPAR) and also CB1R are localized in the nucleus, where they shuttle from/to the cytosol in a ligand-dependent manner.

In addition to these receptors, the ECS is composed of various metabolic enzymes. The eCBs are produced "on demand" from membrane lipid precursors by multiple biosynthetic pathways. These bioactive lipid molecules are synthetized when and where needed and have important roles in physiological and pathophysiological conditions. AEA and 2-AG metabolism occurs through distinct routes that can overlap, of which several have been described in detail. The well-known view is that AEA is synthesized from membrane phospholipid precursors mainly by the action of N-acyl-phosphatidylethanolamine-specific phospholipase D (NAPE-PLD). By contrast, 2-AG is mainly synthesized by 2 diacylglycerol lipase enzymes, DAGLα and DAGLβ. The eCB-mediated effects are terminated by their fast degradation, mainly through the hydrolysis of AEA by the fatty acid amide hydrolase (FAAH) and of 2-AG by the monoacylglycerol lipase (MAGL). Besides these hydrolytic routes, AEA and 2-AG can also be oxidized by cyclooxygenase-2, distinct lipoxygenases, or cytochrome P450, all present in most tissues of the body. Interestingly, AEA and 2-AG oxidative by-products can also produce biological activity that may be mediated by different receptors.

2. Cannabis, the ECS, and the visual system

Besides the scleral vasodilation effect (also known as "red eye") of marijuana and the reduction of intraocular pressure [4, 5], the functional effects of cannabinoids on vision are still not well identified. A case study interviewing eight recent abstinent high-potency heavy cannabis smokers (approximately 56 g per month according to Ref. [6]) reported several categories of visual disturbances [7]. These included visual distortions, biased perception of distance, illusions of movement for stationary and moving objects, color intensification of objects, dimmed

color, dimensional distortion, and blending of patterns and objects [7]. These visual illusions were also experienced by five patients with a history of previous use of marijuana [8]. Interestingly, pupil size, measured with a millimeter rule under constant illumination with eyes focused on an object at constant distance, is not changed after smoking marijuana [9, 10]. It has been shown that eCBs are present in ocular tissues, including the ciliary body, iris, choroid, as well as trabecular meshwork, but not on the lens [3, 5, 11–13]. Hence, eCBs may play an important role in eye function (such as regulation of intraocular pressure) under different normal and pathological conditions [14]. Furthermore, cannabis causes impaired performance in tests that require fine psychomotor control such as tracking a moving point of light on a screen [15, 16]. THC increases the time course of glare recovery by several seconds (5–10%) only at low contrast [16]. Higher doses of THC can produce side effects, including blur vision [17], double vision, and vision dimness [18]. Numerous reports claim that smoking marijuana improves dim light vision [19–21]. Acute consumption of marijuana reduces the Vernier and Snellen acuity, alters color discrimination, increases photosensitivity, and decreases dark adaptation [19, 21, 22]. No significant effect has been observed on static visual acuity [15] after consumption of THC with alcohol, although there was a marked reduction in acuity of moving targets when coordinated eye movements were required [23]. Binocular depth inversion is reduced in regular cannabis users while depth perception is not affected [23, 24]. Dronabinol, a synthetic THC, impaired binocular depth inversion and the top-down processing of visual sensory data [23]. Testing the visual functions by the use of steady-state visual evoked potential and electroencephalography over the occipital lobe suggests a disruption of later-stage visual processing in regular users [25].

3. Retinal anatomy and function in monkeys

3.1. The vervet monkey (*Chlorocebus sabaeus*)

The extensive resemblance of the nonhuman primates to *Homo sapiens* in various aspects, from genome sequence and molecular pathways to physiology and cognition, makes them the closest laboratory model to humans that cannot be approximated by any other animal model. Among the monkeys, old-world monkeys are the closest to the human physiology and behavior, after the apes. Old-world monkeys show more interspecies brain anatomy similarity compared with humans and apes [26]. Although the monkey brain is smaller, it has nonetheless a similar anatomical organization. For example, its visual system also comprises a retina, geniculostriate system, and the "what" (ventral) and "where" (dorsal) pathways, as in humans. The vervet monkey, or green African monkey, is an old-world monkey from the Cercopithecidae family native to Africa that 23 million years ago diverged from the hominoid family, and its genome is 96% homologous with man [27]. Our laboratories have been using vervets for many years now to particularly study the expression and function of the ECS in the retina and visual system. Like all the other old-world monkeys, vervets are medium to large size, have a tail with prehensile nerve ending, and are omnivorous with preference to plant matters. The St-Kitts vervet monkeys were imported from Senegambia in the seventeenth century [28–30]. Vervet monkeys are progressively chosen for biomedical research with a

second citation record among the nonhuman primates after rhesus macaque [26]. Regarding the visual system, old-world monkeys have a foveal binocular vision with laminated retina with a high cone density that decreases with eccentricity and trichromatic color vision. The organization of the retinal mosaic has an impact on visual functions, the center being largely involved in visual acuity, color-coding, and photopic sensitivity (cone vision), whereas the periphery is more concerned with scotopic functions (rod vision) [31, 32]. Vervets also have a six-layered dorsal lateral geniculate nucleus (dLGN) and a laminar organization of the visual cortex similar to that seen in other old-world monkeys and humans [33, 34].

3.2. Description of the monkey retina

The fovea is a small central pit present in the surface of the retina in many types of fish, reptiles, and birds. Among mammals, primates are the only species with a *fovea centralis* [35]. The structure of the fovea can be slightly different in some types of animals. In some animals, the inner cell layers of the fovea may only show a reduced thickness, and in other animals, the fovea may have a complete absence of the same inner cell layers. In monkeys, cone photoreceptors line the base of the foveal pit and the other cells are displaced away from the foveal region. The fovea is at the intersection of retinal and optical axis of the globe and is the area of the most acute vision. The thickness of the retina is reduced in the fovea because the photoreceptor cell synapses and the inner retinal neurons are displaced peripherally from the foveal center. Cones are concentrated in the fovea; its center is free of rods. Peripherally, the number of rods increases, reaching a maximum at the perimeter of the fovea [36]. The diameter of the fovea in humans is 1.5 mm, and the central part, which is free of the retina inner layers, is 0.35 mm across and is called *foveola*. The tissue between the *foveola* and foveal rim, wedge-like in cross section, is called the *clivus* or foveal slope. The axons or outer fibers of the foveal rod and cone cells are elongated and form an additional layer, the "fiber layer of Henle," between the outer nuclear and outer plexiform layer in the periphery of the foveal area (**Figure 1**). The foveal photoreceptor cells, including

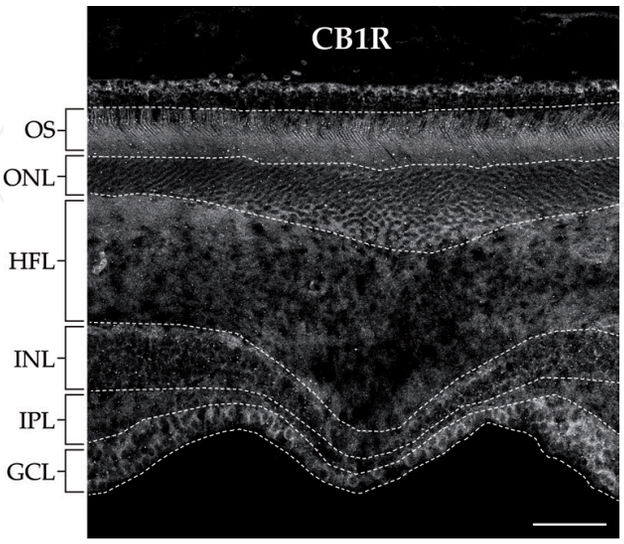

Figure 1. CB1R expression in the monkey fovea centralis. Confocal micrograph of a cross-section retina in the foveal pit immunolabeled for CB1R. OS, photoreceptor outer segments; ONL, outer nuclear layer; HFL, Henle fiber layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Scale bar = 75 μm.

the Henle fibers, contain the yellow pigments zeaxanthin and lutein [37]. The area surrounding the fovea has a distinct tint. It is called *macula lutea*, the yellow spot. The words "macula" and "fovea" are frequently used as synonyms. Blood is supplied to the neuroretina, excluding photoreceptor cells, via retinal vessels. The large arteries and veins lie within the nerve fiber layer. Ascending arteries penetrate into the retinal tissue. Two flat beds of capillaries spread between the perikarya of horizontal cells and amacrine cells, at the outer and inner margins of the internal nuclear layer. Another network of capillaries supplies the ganglion cell and the nerve fiber layer.

3.3. The retinal endocannabinoid system in monkeys

The retina of many species expresses the ECS, including the tiger salamander, goldfish, mouse, rat, chick, tree shrew, vervet monkey, macaque monkey, and human. The anatomical organization of the retina in these species is obviously different, which makes it difficult to infer what really takes place in humans. For example, mice have a rod-dominated retina specialized for vision in nearly complete darkness, referred as scotopic conditions [38]. Additionally, tree shrews have a cone-dominated retina specialized for vision under well-lit conditions, referred to photopic conditions [39]. Primates, including monkeys, have a duplex retina, a fovea with a high cone density that decreases with eccentricity. Mouse and tree shrew retinas have no fovea compared to primates [40], and, compared to rodents, the retina of tree shrews is similar to primates [41, 42]. Comparative studies on the organization of the retina of different animal species led to the conclusion that ancestral mammals may have already developed cone photopigments [32]. Many components of the ECS have been localized in cone photoreceptors, horizontal, amacrine, bipolar, and retinal ganglion cells in the central and peripheral retina of vervet monkeys (**Figure 1**; [43]).

Compared to rodents, the retina of primates including monkeys and humans has the unique characteristic to have a duplex retina with a cone-dominated fovea [44]. As part of the brain, this highly organized tissue processes visual information in parallel channels. While the input retina consists of only 2 types of photoreceptors (rods sensitive to 1 wavelength of light and cones selective to 3 different wavelengths), the output retina contains more than 20 types of ganglion cells [45, 46]. The primate retina exhibits a strikingly high expression of CB1R, the main cannabinoid-binding protein responsible for the marijuana psychotropic effects. Cones of the central retina abundantly expressed CB1R. The vertical glutamate pathway (cone photoreceptors-bipolar cells-ganglion cells) also heavily expresses CB1R. While the functional importance of retinal CB1R is supported by anatomical data in rodents and primates, evidence for a role of eCBs in synaptic signaling is provided by *in vivo* ERG experiments on vervet monkeys. The presence of CB1 receptors in the retina of many species has been reported [47]. The modulatory effects of cannabinoids, acting on CB1 receptors, at all stages of retinal processing have also been described (For review, see [48]). Furthermore, the cannabinoid receptors (**Figure 2**) and related enzymes (**Figure 3**) are expressed in the mouse, tree shrew, vervet monkey, and macaque monkey retina [49]. More specifically, the ECS is present throughout the monkey retina, from the foveal pit to the periphery, suggesting that it may play a role in retinal function.

3.4. Electroretinography in monkeys

Electroretinography (ERG) is a noninvasive ocular test that measures the electrical responses of various cell types in the retina, including rod and cone photoreceptors, horizontal cells, bipolar

cells, amacrines cells, ganglion cells, and Müller cells. This diagnostic tool can objectively evaluate retinal function in clinical and research settings. It is well-known that the monkey eye anatomy and physiology are similar to those of humans, making it the preferred nonhuman primate animal model for testing ocular effects. Given that the technical and procedural aspects of the human

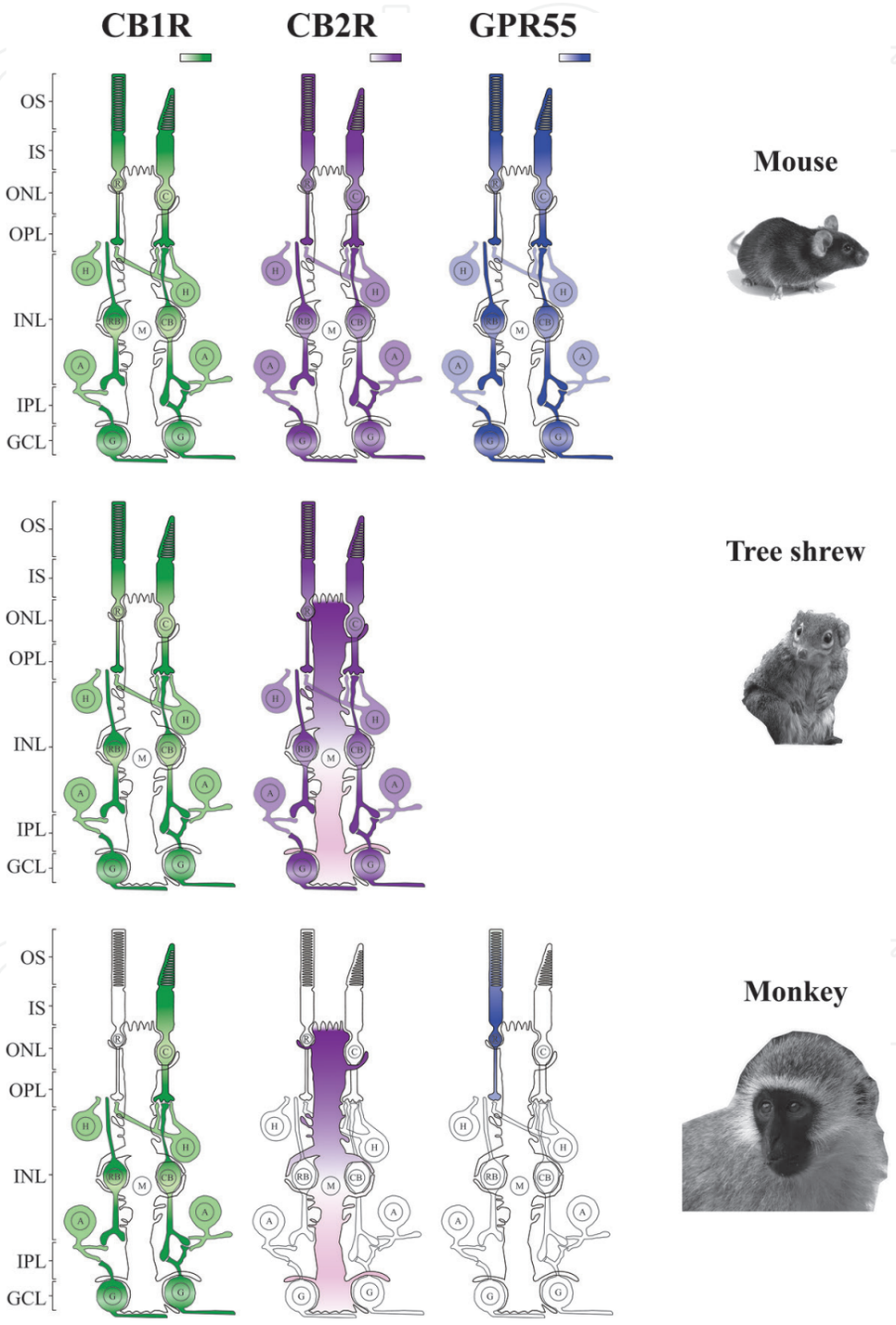

Figure 2. Mapping of the receptors CB1R, CB2R, and GPR55 in the mouse, tree shrew, and monkey retina. These receptors are differently expressed in the retina of these mammals. These results are compiled from several published articles [43, 47–54]. OS, photoreceptor outer segments; IS, photoreceptor inner segments; ONL, outer nuclear layer; ONL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

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protocol have been standardized by the International Society for Clinical Electrophysiology of Vision [58], the ERG can be routinely used to assess toxicity (potential global neurotoxicity induced by drugs in primates) on retinal function. With this standard method, a tremendous amount of

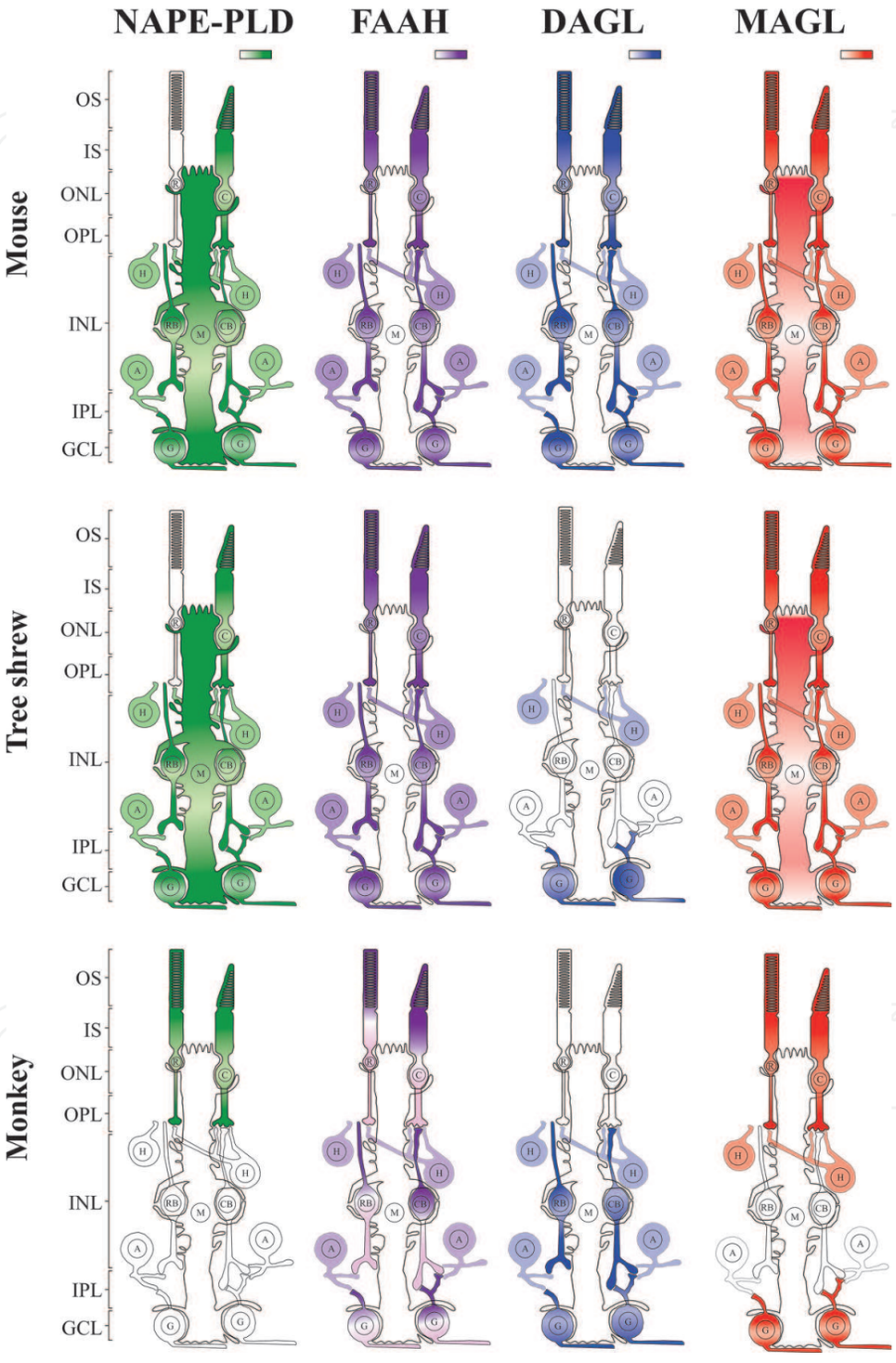

Figure 3. Localization of the cannabinoid-related enzymes in the mouse, tree shrew, and monkey retina. NAPE-PLD, FAAH, DAGL, and MAGL expressions have some similarities and differences in the retina of these mammals. These results summarize results published in several articles [43, 49, 50, 55–57]. OS, photoreceptor outer segments; IS, photoreceptor inner segments; ONL, outer nuclear layer; ONL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.

data can be accumulated for eye physiopathology evaluation. This noninvasive, nonpainful, nondamaging technique does not induce undesirable effects to the eye. Numerous studies of ocular toxicity in macaque monkeys have produced a standard ERG protocol for the assessment of retinal function [59]. This method allows independent testing of rod and cone systems. If a low-intensity flash stimulus is conveyed to the dark-adapted retina, the rod system is stimulated. If a flash stimulus is conveyed to the light-adapted retina, the cone system is targeted. When strong flash stimuli are elicited, the retina electrical responses will produce an ERG waveform comprising an initial corneal-negative deflection derived from rods and cones, the a-wave, followed by a corneal-positive deflection derived from the inner retina (predominantly Müller and ON-bipolar cells), the b-wave. A standardized experimental protocol was developed in our laboratory and used to study the role of the ECS on retinal function in vervet monkeys [60]. The mobile experimental setup consisted of placing ERG-Jet electrodes in both eyes for simultaneous recordings (**Figure 4**). In dark-adapted conditions, dim flashes activate the scotopic system (rod pathway) and photopic flashes evoke a mixed response (rod and cone pathways). In light-adapted conditions, flashes produce only a photopic system response (cone pathway).

CB1R, CB2R, and GPR55 play an important role in retinal function. The function of the cannabinoid receptors in the retina has been highlighted in ERG studies in adult mice [53] and vervet monkeys [61, 62]. Cécyre et al. [53] demonstrated a significant change in the ERG a-wave of the CB2R knockout dark-adapted mouse but not in CB1R knockouts compared to wild types. They concluded that CB2R is likely to play a greater role in mouse retinal processing than CB1R. In monkeys, we have recently reported a significant increase in the b-wave component of the scotopic and photopic ERG after a blockade of both CB1R and CB2R with their specific antagonists [61]. This variation can be due to the different pattern of expression and specialization of the ECS in the monkey. We also reported that GPR55 may play an instrumental role in mediating scotopic vision, because it is exclusively expressed in rods [52] and it modulates specifically scotopic retinal function [62]. **Figure 5** summarizes the ERG effects

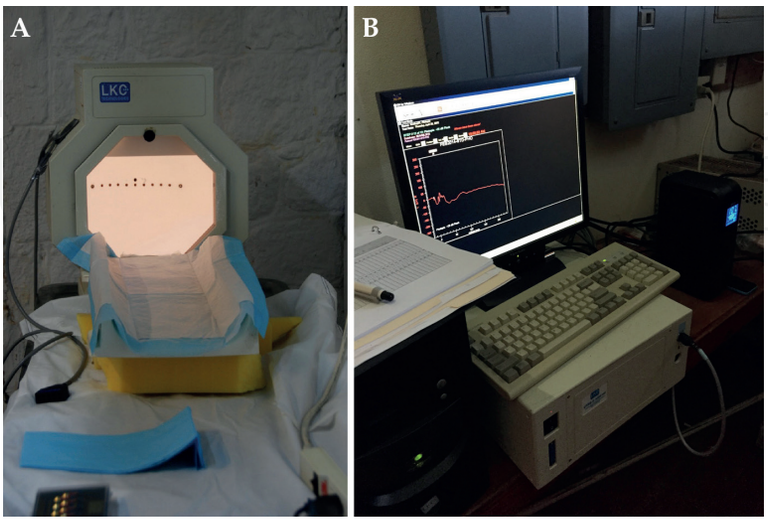

Figure 4. Experimental setup for ERG recordings in vervet monkeys. (A) The ganzfeld allows us to illicit full-field flashes. (B) The ERG machine (UTAS-E3000) is linked to a computer.

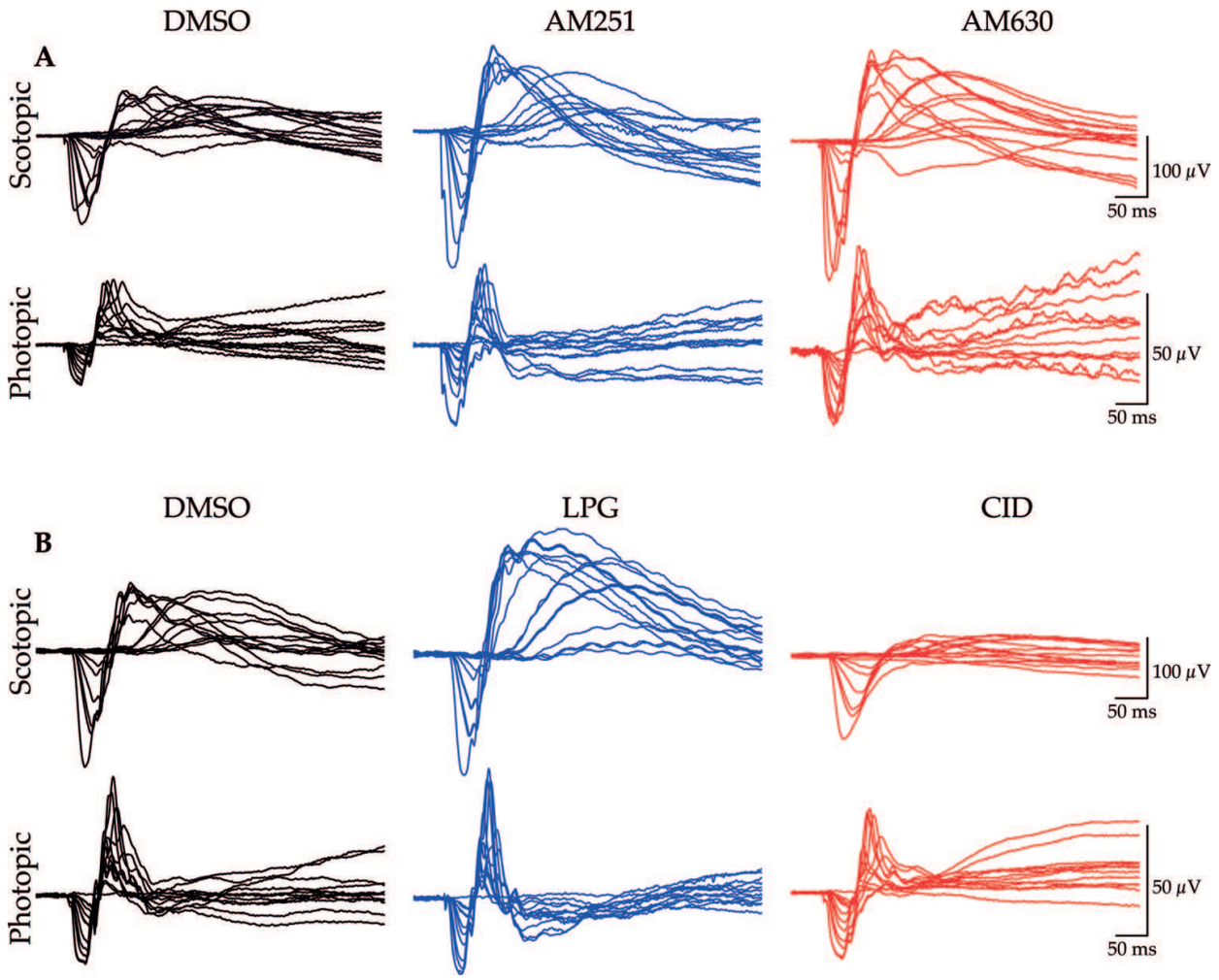

Figure 5. The effect of modulating CB1R, CB2R, or GPR55 in the monkey retina. (A) The intravitreal injection of AM251, an inverse agonist of CB1R, or AM630, an inverse agonist of CB2R, causes an increase of the scotopic and photopic responses compared to the vehicle, dimethyl sulfoxide (DMSO). (B) The intravitreal injection of lysophosphatidylglucoside (LPG), an agonist of GPR55, causes an increase of the scotopic response, but not of the photopic response. Conversely, the intravitreal injection of CID16020046 (CID), an antagonist of GPR55, causes a decrease of the scotopic response, but not of the photopic response.

obtained in vervet monkeys. This body of evidence (the anecdotal reports, the anatomical localization of the ECS, and its functional implications) indicates that eCBs are involved in shaping retinal responses to light and suggests it plays a crucial role in visual processing.

3.5. The eCB signaling pathways in the monkey retina

The presence of CB1R in the neuroretina (in the vertical pathway consisting of photoreceptors, bipolar cells, and ganglion cells), of CB2R in the major glial element of the retina (Müller cells), and of GPR55 in rod photoreceptors suggest differential retinal function. Furthermore, localization of the metabolic enzymes suggests that eCBs are synthesized and released in the synapse surrounding the neurons from which they are released. They therefore act locally on adjacent retinal cells [63]. This could in turn influence, directly (through CB1R or GPR55) or

indirectly (through CB2R), the release of glutamate, the main neurotransmitter of the retinal vertical pathway. After the ligands are produced, many ionic channels such as K^+ and Ca^{++} are modulated after activation of the cannabinoid receptors.

The suggested hypothetical function of the ECS in the monkey retina may be as follows. In photopic conditions, when cones are stimulated by light, the ionic channels are inhibited, a process known as the "*inhibition of the retinal dark currents*." The resulting phototransduction reduces the glutamate release in the synapse and propagates an evoked potential to bipolar cells. Given the localization of the metabolic enzymes in monkeys (**Figure 3**), the same bipolar cells may be the main source of eCB production that will act in a retrograde manner and activate CB1R located in cone pedicles, thus regulating glutamate release. This eCB production will also synthesize 2-AG that will activate CB2R in Müller cells, thus modulating potassium spatial buffering throughout the retina. The activation of CB2R coupled to $G_{i/o}$ will reduce the levels of cyclic AMP and PKA ([64] for review; [65]). Given that PKA activates $K_{IR}4.1$ channels in Müller cells [66], CB2R will play a role by negatively modulating potassium.

In scotopic conditions, the synaptic terminals of rods release a large quantity of glutamate. This glutamate binds to mGluR6 receptors located on the dendrites of ON rod bipolar cells [67]. Activation of GPR55 by its endogenous agonist (lysophosphatidylglucoside [LPG]) will stimulate the $G_{\alpha 13}$, RhoA, ROCK, and PLC cascade to open Na^+/Ca^{++} channels, induce a membrane depolarization, and, finally, modify the scotopic ERG [52, 62].

These proposed mechanisms of action for the photoreceptor-bipolar cell synapse are illustrated in **Figure 6** and could also take place in other synapses in the primate retina. However, even though great efforts are deployed to understand the precise function of the retinal ECS, behavioral studies are crucial to clearly establishing its role in vision.

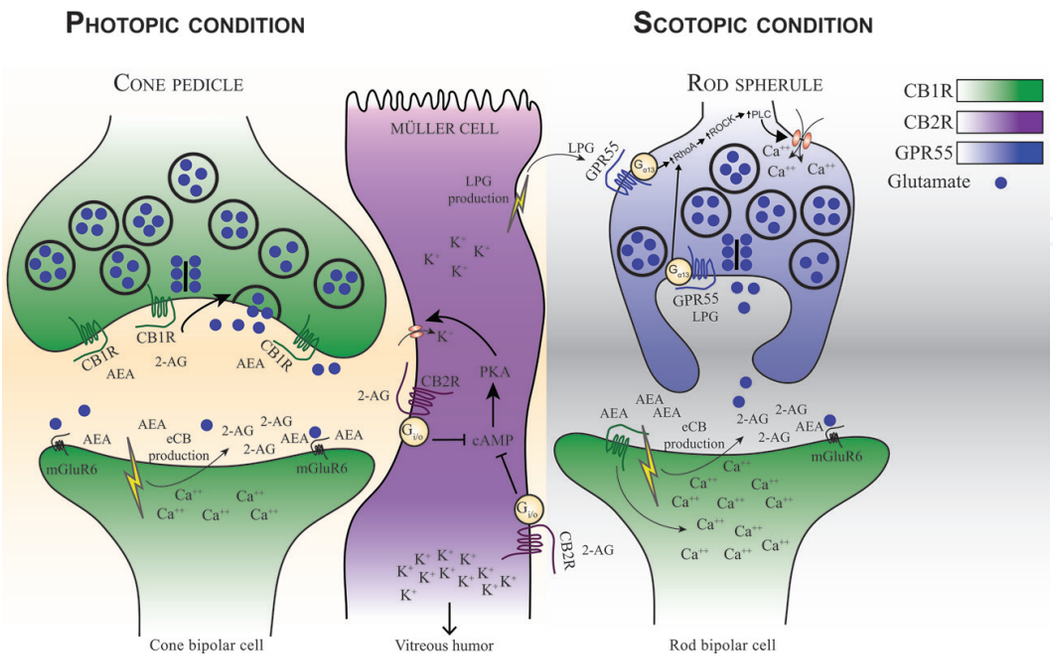

Figure 6. Schematic illustration of the hypothetical function of CB1R, CB2R, and GPR55 in the monkey retina. See text for details.

4. Beyond the retina, in the visual thalamus and visual cortex

Multiple studies have studied the expression of the ECS in the thalamus of rodents and primates, but only one has thoroughly examined the expression of CB1R, NAPE-PLD, and FAAH in the dorsal lateral geniculate nucleus of vervet monkeys [34]. One study has characterized the physiological effects of CB1R-mediated activity in the visual thalamus of rats [68]. Using single-unit extracellular recordings at the level of the thalamus, CB1R activation affected two cell populations: one exhibiting excitatory effects (28%) and the other inhibitory effects (72%) [68]. When AM251 (inverse agonist of CB1R) was added, these actions were blocked. The authors concluded that CB1R in the rat thalamus acts as a dynamic modulator of visual information. Furthermore, the CB1R system is present in the lateral geniculate nucleus of monkeys [34]. The presence of CB1R in the dLGN of vervet monkeys could have a higher impact on visual processing, compared to what is found in rats [68], due to its complex laminar structure and increased retinal inputs. The results reported by Javadi et al. [34] are in accordance with these electrophysiological findings and revealed that this neuromodulation of CB1R receptors may be due to their expression in the dLGN. Even though the expression of CB1R and FAAH is more abundant in magnocellular layers, they are nonetheless present in the parvocellular layers of the dLGN. The high expression of CB1R in the magnocellular layers may explain some of the behavioral effects of cannabis and cannabinoids associated with the integrity of the dorsal visual pathway (visual-spatial localization and motion perception) [34]. If the ECS plays any role in color perception, it should be through chromatic properties of parvo cells and retinal cones. Both of these visual system components express CB1R. Conjointly, one of the most frequently reported cannabis effects is more intense and brighter colors [7, 69].

The visual cortex is important to the conscious processing of visual information. The exact localization of the ECS in higher visual structures remains somewhat unclear. Several published reports describe the effects of cannabinoids on visual perception (thresholds of light detection, glare recovery, and color vision). The localization of the ECS within the higher order visual areas responsible for these changes in perception is starting to be revealed. As noted above, there has been evidence for central effects of cannabinoid use in vision by binocular depth inversion technique and EEG recordings of the occipital cortex [24, 25]. Yoneda and colleagues reported that the expression and localization of CB1R in the visual cortex of the mouse is regulated during the development and through visual experiences. In mouse, expression of CB1R in deep layers of V1 decreased after dark rearing from birth to P30. However, 2 days of monocular deprivation upregulated the localization of CB1R in inhibitory nerve terminal in deep layers [70]. It has also been reported that CP55940, a full agonist of CB1R and CB2R, decreases the electroencephalogram power, and the local field power and coherence, in V1 and V2 in macaque monkeys [71].

5. The ECS and visual disorders

The ECS modulates many neurotransmission processes in the central nervous system. In fact, numerous recent researches raised the impact of metabotropic and ionotropic receptors on

neurological diseases. Studying modulator systems like the monoaminergic, purinergic, and cholinergic systems may reveal the pathophysiology of many disorders. Opioid and nicotinic receptors were generally analyzed in order to treat drugs of abuse, morphine and nicotine. Conversely, the study of cannabinoid receptors is not primarily to treat addiction to marijuana but has a much broader role. Indeed, CB1R is the most abundant metabotropic receptor in the CNS [72]. These receptors together with their ligands and related enzymes constitute a goldmine in the chase of finding therapeutic targets against many visual pathologies. For instance, blindness and visual impairment are relatively refractory to most of the current drugs, emphasizing the importance of identifying a novel site of action for pharmacological treatments. Accordingly, modulation of the cannabinoid system remains potentially a new therapeutic approach. This could be performed at several levels. For endogenous cannabinoids, it would be a modulation of their synthesis, release, cellular uptake, metabolism, or interactions with cannabinoid receptors. Biochemical imbalances in the ECS in visual structures may cause or exacerbate pathological disorders, such as glaucoma ([73] for review), diabetic retinopathy, and age-related macular degeneration [14]. The variation of the content of eCBs in these diseases suggests that they play a crucial role in ocular homeostasis. Indeed, patients with glaucoma have a decrease in 2-AG levels in the ciliary body [12]. In age-related macular degeneration (AMD) patients, 2-AG levels are amplified in the iris and AEA is also increased in the retina [14]. The same pattern of augmentation of AEA was observed in choroid, ciliary body, and cornea of the AMD patients [14]. Moreover, many recent studies investigating the role of cannabinoids in visual development have shown that CB1R is transiently expressed throughout development of the chick and rat retinas. Nevertheless, cannabis and cannabinoids as therapeutic agents have not yet been unequivocally established. Targeting preferentially retinal cannabinoid receptors to avoid unwanted psychotropic effects is a new interesting avenue requiring further investigation.

6. Future research directions

There is now strong evidence that suggests that the ECS plays a significant role in regulating visual function. However, several important questions remain to be answered. First, there is a need to define the exact mechanisms by which eCB production in the retina is regulated. Then, we need to determine if eCB production is influenced by classical calcium-regulating hormones, cytokines, and mechanical loading. Further research is also necessary to define the exact signaling pathways used by cannabinoid receptors to regulate retinal cell activity. There is evidence that CB1R regulates photoreceptors, horizontal cells, bipolar cells, amacrine cells, and ganglion cell activity through a cAMP-mediated pathway (for review, see [48]), but little is known on how cannabinoids regulate global retinal activity. There have been major inconsistencies between different studies with regard to the expression and function of the ECS in the retina and the brain. These inconsistencies may be due to the nonspecificity of the available cannabinoid receptor ligands that are thought to be specific for CB1R or CB2R but can actually bind to GPR55 [74–76]. A further area of research that remains to be explored is to determine how cannabinoid receptors exert their effects on vision, through a neuronal or glial mechanism. This is clinically relevant since if the glial effects were predominant, it

may be possible to develop agonists of these receptors that do not affect neuronal function but could favorably influence visual function without causing adverse psychotropic neuronal effects. The outcome of these studies will greatly enhance our understanding of the role of the ECS in vision and encourage the development of new treatments for visual disorders based on targeting the ECS.

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