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# THE ENDOCANNABINOID SYSTEM WITHIN THE DORSAL LATERAL GENICULATE NUCLEUS OF THE VERVET MONKEY

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**Abstract—The endocannabinoid system mainly consists of cannabinoid receptors type 1 (CB1R) and type 2 (CB2R), their endogenous ligands termed endocannabinoids (eCBs), and the enzymes responsible for the synthesis and degradation of eCBs. These cannabinoid receptors have been well characterized in rodent and monkey retinae. Here, we investigated the expression and localization of the eCB system beyond the retina, namely the first thalamic relay, the dorsal lateral geniculate nucleus (dLGN), of vervet monkeys using immunohistochemistry methods. Our results show that CB1R is expressed throughout the dLGN with more prominent labeling in the magnocellular layers. The same pattern is observed for the degradation enzyme, fatty acid amide hydrolase (FAAH). However, the synthesizing enzyme N-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD) is expressed homogenously throughout the dLGN with no preference for any of the layers. These proteins are weakly expressed in the koniocellular layers. These results suggest that the presence of the eCB system throughout the layers of the dLGN may represent a novel site of neuromodulatory action in normal vision. The larger amount of CB1R in the dLGN magnocellular layers may explain some of the behavioral effects of cannabinoids associated with the integrity of the dorsal visual pathway that plays a role in visual-spatial localization and motion perception.**


**Key words: CB1R, FAAH, NAPE-PLD, dLGN, monkey, cannabinoid receptors.**

## INTRODUCTION

The physiological and psychological effects of phytocannabinoids, the active components of the cannabis plant, can be detected almost everywhere in the body due to their actions on specific receptors: mainly the cannabinoid receptors type 1 (CB1R) and type 2 (CB2R). Cannabinoid receptors are membrane receptors principally coupled to inhibitory G-proteins that modulate the release of neurotransmitters (Piomelli, 2003; Gómez-Ruiz et al., 2007). They mediate biological functions not only via the exogenous cannabinoids, but also via eCBs such as N-arachidonoylethanolamide (anandamide or AEA) and 2-arachidonoylglycerol (2-AG). Unlike the classical neurotransmitters, eCBs are synthesized "on demand" by catalyzing the release of N-acylethanolamines (NAEs) from N-acyl-phosphatidylethanolamine (NAPE) by specific enzyme, like N-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD) or from arachidonic acid via diacylglycerol lipase enzyme (DAGL). The eCBs are not accumulated into synaptic vesicles and are rather degraded rapidly by specific enzymes like fatty acid amid hydrolase (FAAH) and monoglycerol lipase (MAGL) (for review, see Deutsch and Chin, 1993).

The localization and function of the molecular components of the eCB system in the central nervous system have been the subject of recent research. In fact, the role of the eCB system in learning, memory, neuroprotection and visual processing is essentially due to the modulation of neurotransmitter release by the presynaptic location of CB1R (Di Marzo et al., 1998; Straiker et al., 1999a). CB1R expression is found in the hippocampus, prefrontal cortex, cerebellum and basal ganglia of rodents (Herkenham et al., 1991) and primates (Eggan and Lewis, 2007). It is expressed in glutamatergic and GABAergic neurons throughout the central and peripheral nervous systems (Egertová and Elphick, 2000). In the visual system, CB1R and FAAH have been localized in cone photoreceptors, horizontal, amacrine, bipolar, and retinal ganglion cells in the central and peripheral retina of vervet monkeys (Bouskila et al., 2012). CB1R is also found in the human retina (Straiker et al., 1999b).

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*Abbreviations:* AEA, anandamide; CB1R, cannabinoid receptor CB1; DAB, 3,3′-diaminobenzidine; DAGL, diacylglycerol lipase enzyme; dLGN, dorsal lateral geniculate nucleus; eCB, endocannabinoid; ERG, electroretinogram; FAAH, fatty acid amide hydrolase; GABA, GABAergic cell; GFAP, glial fibrillary acidic protein; IR, immunoreactivity; MAGL, monoglycerol lipase; MT, middle temporal; MST, medial superior temporal; NAE, N-acylethanolamines; NAPE, N-acyl-phosphatidylethanolamine; NAPE-PLD, N-acyl-phosphatidylethanolamine phospholipase D; NDS, normal donkey serum; PBS, phosphate-buffered saline; SEM, standard error of the mean; VGLUT1, vesicular glutamate transporter 1; 2-AG, 2-arachidonoylglycerol.

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Earlier studies reported that cannabis could affect several visual functions, such as photosensitivity (Adams et al., 1978), visual acuity (Moskowitz et al., 1972; Adams and Brown, 1975), color vision (Dawson et al., 1977), ocular tracking (Flom et al., 1976), binocular depth inversion, and stereoscopic vision (Emrich et al., 1991; Leweke et al., 1999; Semple et al., 2003). Some case studies later claimed other visual effects of cannabis such as visual distortions, altered perception of distance, illusions of movement in stationary and moving objects, color intensification of objects, dimensional distortion and blending of patterns and objects (Levi and Miller, 1990; Lerner et al., 2011). Given the localization of CB1R in the central retina, from cones to ganglion cells, it is reasonable to assume its implication in these visual manifestations.

In homogenates of rodent thalamus, high levels of AEA (Felder et al., 1996) and FAAH (Egertová et al., 2003), as well as an elevated cannabinoid receptor/G-protein amplification ratio (Breivogel et al., 1997) have been found. Also, using immunohistochemistry, moderate to low levels of CB1R expressions have been found in the thalamus of rats (Egertová et al., 1998; Tsou et al., 1998; Moldrich and Wenger, 2000), non-human primates (Ong and Mackie, 1999) and humans (Glass et al., 1997) without focusing on dorsal lateral geniculate nucleus (dLGN). However, there is no study, to our knowledge, that has thoroughly studied the expression of the eCB system in this retino-recipient primary thalamic relay of the primate. Similar to apes and humans, the dLGN of vervet monkeys consists of six layers. The first two ventral layers, the magnocellular layers, receive input from large ganglion cells (rod signals) and are necessary for the perception of movement, depth and small difference in brightness. The four dorsal layers, parvocellular layers, receive input from small ganglion cells of the retina (cone signals) and play a role in color and form perception. These layers are well separated by an inter-laminar zone called koniocellular layers that contribute to short-wavelength "blue" cones (Xu et al., 2001). Given the expression and localization of CB1R in the retinal mosaic, we expect to find this receptor in the optic nerve and the dLGN layers.

## EXPERIMENTAL PROCEDURES

### Animals

Monkey tissues were obtained from four adult vervet monkeys (*Chlorocebus sabaeus*). The monkeys were part of Dr. Ptito's and Dr. Palmour's research project that was approved by McGill University Animal Care and Use Committee. The animals were born and raised under an enriched natural environment in the laboratories of the Behavioral Sciences Foundation (St-Kitts, West Indies), a facility recognized by the Canadian Council on Animal Care (CCAC). The experimental protocol was reviewed and approved by the local Animal Care and Use Committee (University of Montreal) and the Institutional Review Board of the Behavioral Science Foundation.

### Tissue preparation

Each animal was sedated with ketamine hydrochloride (10 mg/kg, i.m.) and euthanized by an overdose of intravenously administered sodium pentobarbital (25 mg/kg), followed by transcardial perfusion of 0.1 M phosphate-buffered saline (PBS) (pH 7.4). The brains were then removed, blocked and flash-frozen in an isopentane bath cooled in a dry ice chamber and maintained at −80 °C. The blocks were cut along the coronal plane in 20-μm sections at −18 °C on a Leica CM3050S cryostat and mounted onto gelatinized subbed glass slides. The slide-mounted tissue sections were stored at −80 °C until further histological processing.

### Immunohistochemistry (DAB)

At least one slide-mounted 20-μm fresh-frozen tissue section per animal was selected from A6 to A9, at a level where the lamination of the dLGN is the clearest and thawed at room temperature. A hydrophobic barrier was created surrounding the slides, using PAP pen (Vector, Burlingame, CA, USA) to keep staining reagents localized on the tissue section. Sections were fixed with 70% ethanol solution for 15 min, followed by two 5-min rinses with 0.1 M Tris buffer, pH 7.4/0.03% Triton X-100. To block the endogenous peroxidase activity, sections were washed with 0.3% hydrogen peroxide in PBS for 15 min. Following three times 5-min PBS-triton rinse, sections were blocked for 60 min with a solution of 10% normal donkey serum (NDS) and 0.1 M Tris buffer/0.5% Triton. Each section was incubated overnight at room temperature with primary antibodies (Table 1) diluted in the blocking solution. The next day, sections underwent three 10-min PBS-triton washes, followed by incubation in a secondary antibody solution (biotinylated donkey anti-rabbit antibody diluted 1:200 in blocking solution) for 2 h. After three consecutive 10-min washes with PBS-triton, the sections were incubated for 1 h in an avidin–biotin-conjugated horseradish peroxidase (Vectastain ABC kit, Burlingame, CA, USA) solution (1:500). Following three subsequent 10-min washes in PBS-triton, the sections were treated with a 3,3′-diaminobenzidine (DAB) substrate. After rinsing in PBS-triton three times for 5 min each, sections underwent dehydration in graded ethanol steps, cleared in xylene, and cover-slipped with Permount mounting media (Fisher Scientific; Pittsburgh, PA, USA). Sections were examined on a Leica DMRB under bright field illumination.

### Immunofluorescence

Double and triple labeling of the brain tissues were performed according to previously published methods on the vervet monkey retina (Bouskila et al., 2012; Bouskila et al., 2013a,b). Briefly, sections were post-fixed for 15 min in 70% ethanol, rinsed two times for 5 min in 0.1 M Tris buffer, pH 7.4/0.03% Triton and blocked for 90 min in 10% NDS and 0.1 M Tris buffer/0.5% Triton. Sections were incubated overnight at room temperature