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# Immunohistochemical distribution of the cannabinoid receptor 1 and fatty acid amide hydrolase in the dog claustrum

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ABSTRACT

Cannabinoid receptor 1 (CB1R) and fatty acid amide hydrolase (FAAH) are part of the endocannabinoid system (ECB) which exerts a neuromodulatory activity on different brain functions and plays a key role in neurogenesis. Although many studies have reported FAAH and CB1R expression in the brain of different animal species, to the best of our knowledge they have never been described in the canine claustrum. Claustrum samples, obtained from necropsy of four neurologically normal dogs, were formalin fixed for paraffin embedding. Sections were either stained for morpho-histological analysis or immunostained for CB1R and FAAH. Analysis of adjacent sections incubated with the two antisera showed a complementary labeling pattern in the claustrum, with CB1R antibody staining fibers while anti-FAAH antibody stained cell bodies and the proximal portion of dendrites; this particular anatomical relationship suggests a retrograde endocannabinoid action via CB1R. CB1R and FAAH complementary immunostaining and their cellular localization reported here provide the first anatomical evidence for existence of the ECB in the dog claustrum.



## 1. Introduction

The herbaceous plant *Cannabis sativa* (marijuana) contains an array of pharmacologically active compounds, including the cannabinoids known for their psychoactive effects. Among these phytochemicals is $\Delta^9$-tetrahydrocannabinol the chief psychoactive component in marijuana. This phytocannabinoid exerts its action by binding to cannabinoid receptor 1 (CB1R), the most abundant G-protein-coupled receptor in brain (Covey et al., 2014). CB1R was first identified by Devane et al. (1988) and later cloned by Matsuda et al. (1990). A second cannabinoid receptor (CB2R), mainly expressed in immune cells, was subsequently identified by Munro et al. (1993). In the central nervous system (CNS) CB1R receptor is activated by the endogenous cannabinoids (endocannbinoids; ECs) anandamide (Devane et al., 1992) and 2-arachidonoyl-glycerol (2-AG) (Mechoulam et al., 1995). Anandamide and 2-AG are degraded by fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MAGL), respectively (Di Marzo et al., 1999; Di Marzo et al., 1998; Goparaju et al., 1999; Goparaju et al., 1998). MAGL is mainly localized to the presynaptic axon terminals, while FAAH is a cytoplasmic enzyme localized in somata and dendrites of postsynaptic elements (Dinh et al., 2002; Egertova et al., 2003; Gulyas et al., 2004; Tsou et al., 1998b). Both anandamide and 2-AG are synthesized postsynaptically whereas CB1R is located on presynaptic terminals, suggesting a ECs retrograde release mechanism which suppresses presynaptic neurotransmitter release (Kreitzer and Regehr, 2001; Maejima et al., 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). Termination of the retrograde signal depends on EC uptake from its site of action and degradation by FAAH and MAGL (Ohno-Shosaku and Kano, 2014).

CB1R, ECs and their degrading/synthetic enzymes make up the ECB which exerts a neuromodulatory activity on different brain functions (Moreira and Lutz, 2008; Solinas et al., 2008) and plays a key role in neurogenesis (Prenderville et al., 2015). Moreover, it is well known that the ECB is implicated in a variety of psychological and psychiatric disorders, including drug addiction (Gardner, 2005; Maldonado et al., 2006). The analgesic properties of cannabinoids have been demonstrated in animal models of pain (Rice, 2001).

The topographical distribution of CB1R in the brain has been extensively investigated, with immunohistochemical findings showing its presence in mouse (Cristino et al., 2006) and dog brain (Campora et al., 2012). In a knock-in mouse line genetic

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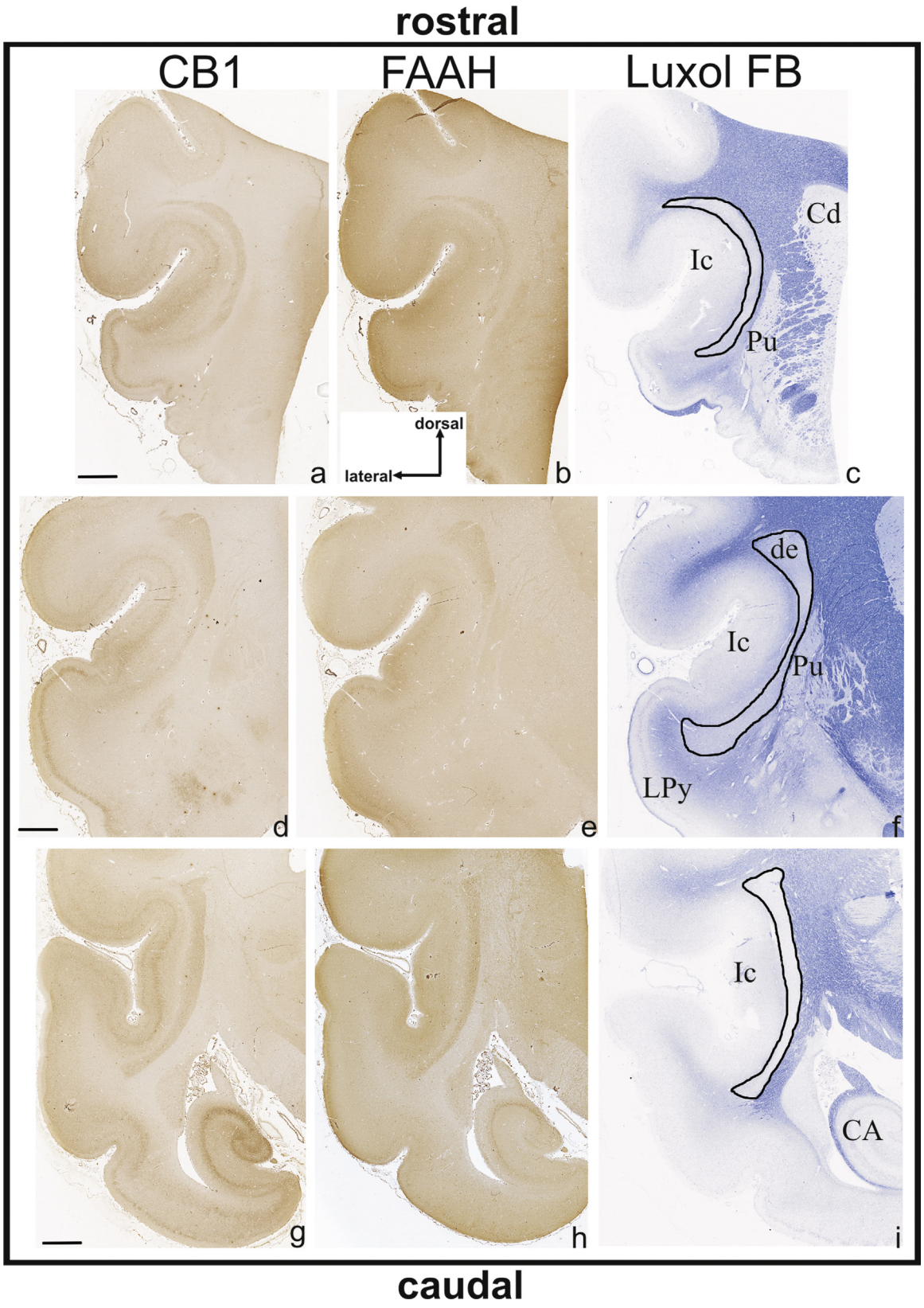


**Fig. 1.** Photomicrographs of immunohistochemical and histological staining of adjacent transverse brain sections. Low magnification image of the CB1R (a, d, g) and FAAH (b, e, h) immunostaining distribution in the left hemisphere including the claustrum. (c, f, i) Low magnification image of a Luxol Fast Blue stained section, with the claustrum outlined in black. CA, hippocampus; Cd, caudate nucleus; de, dorsal enlargement; Ic, insular cortex; LPy, pyriform lobe; Pu, putamen. Scale bars = 2 mm.