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Parvalbumin expression in the claustrum of the adult dog. An immunohistochemical and topographical study with comparative notes on the structure of the nucleus

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ABSTRACT

Although the detailed structure and function of the claustrum remain enigmatic, its extensive reciprocal connection with the cortex suggests a role in the integration of multisensory information.

Claustrum samples, obtained from necropsy of four dogs, were formalin fixed for paraffin embedding. Sections were either stained for morpho-histological analysis or immunostained for parvalbumin (PV). We focused on PV because in cortical and hippocampal areas it is a marker of the fast-spiking interneurons which have an important role in the information transmission and processing. Soma area, perimeter and circularity were considered as morphological parameters to quantitatively group the PV positive somata by k-means clustering.

The histological investigation revealed a superior pyramidoid puddle and a posterior puddle characterized by a "cloud" of neurons in its dorso-lateral part. Immunostaining showed positive somata and fibers throughout the rostro-caudal extent of the dog claustrum, localized principally in the dorsal region. k-Means clustering analysis enabled neuron classification according to size, identifying respectively big (radius = 11.42 ± 1.99 μm) and small (radius = 6.33 ± 1.08 μm) cells. No statistical differences in soma shape were observed. The topographical distribution of PV immunoreactivity suggests that the dog dorsal claustrum might be functionally related to the processing of visual inputs.

Taken together our findings may help in the understanding the physiology of claustrum when compared with anatomical and functional data obtained in other species.

Introduction

The claustrum is a subcortical telencephalic structure present in all the placental mammals investigated so far (Kowianski et al., 1999). Due to its modest size, intricate shape and deep internal location, its anatomy, physiology and ontogenesis is still a matter of debate (Edelstein and Denaro, 2004; Pirone et al., 2012; Mathur, 2014). It has an extensive afferent and efferent network with the cortex, with a special relationship to the visual cortex in the cat (Olson and Graybiel, 1980; Minciacchi et al., 1995) and monkey (Remedios et al., 2010), consequently it has been hypothesized that this structure might be a nervous center where different cortical information are processed and integrated (Crick and Koch, 2005). Crick and Koch (2005) refer to the claustrum as "a conductor coordinating a group of players in the orchestra, the various cortical regions". In line with this view, Smythies et al. (2012, 2014) described the claustrum interneurons as an "interactive gap-junction syncytium".

Nevertheless, a recent anatomical study including various species depicted the claustrum as an irregular structure with many isolated cells islands observed in cetaceans (Baizer et al., 2014). According to these discontinuities the authors argued against the postulated role of the claustrum as a conductor.

The claustral interneurons, as in the cortex (Druga, 2009), seem to play a crucial role in the inhibitory circuits providing a substrate for local information processing (Crick and Koch, 2005).

Calcium-binding proteins (CBps) (calretinin CR, calbindin CB and parvalbumin PV) are considered markers of three non-ovarlapping interneuronal populations (Druga, 2009; Barinka and Druga, 2010). Moreover, a recent study indicates that cortical interneurons can also be classified by their expression of PV, somatostatin, and vasointestinal peptide (Xu et al., 2010).

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Table 1
Details of the sampled dogs.

No.	Breed	Age	Sex	Body weight (kg)	Brain Weight* (g)	Cause of death
1	Dachshund	11	Female	11	64	Intra-abdominal hemorrhage
2	Fox terrier	12	Male	10	66	Peritonitis
3	Mixed-breed	4	Male	14.5	70	Trauma
4	Mixed-breed	9	Male	16	72	Heart failure

* Formalin fixed.

PV is a marker of a specific class of interneurons, the category of fast-spiking neurons, involved in the generation of gamma oscillations which have an important role in the transmission of information between cortical and hippocampal areas (Buzsaki et al., 1983; Whittington et al., 1995; Tamas et al., 2000; Salinas and Sejnowski, 2001; Bartos et al., 2007; Freund and Katona, 2007; Gonzalez-Burgos and Lewis, 2008; Roopun et al., 2008; Cardin et al., 2009; Uhlhaas and Singer, 2010).

The presence of PV-immunoreactive (ir) interneurons, with gap junctions along their dendrites, was described in the cerebral cortex supragranular layer (Fukuda, 2007). In the cerebellar Golgi cells it has been demonstrated that the protein connexin-36 is necessary for electrical synapses (Vervaeke et al., 2010). Gap junctions, thus electrical synapses, are regarded as an important factor of synchronous activity (Galarreta and Hestrin, 2001; Hestrin and Galarreta, 2005). However, connexin-36 or other markers of gap junctions has not been found in the claustrum neurons so far. Interestingly, a decrease of PV-ir interneurons seems to result in a reduction in coordinated neuronal activity during task performance in a rat model of schizophrenia (Lodge et al., 2009). Moreover, a decrease of PV-ir neurons in the central nervous system of patients with Alzheimer's disease has been observed (Satoh et al., 1991). Dogs show many neurological disorders which phenotypically resemble human diseases, thus this species can be used as a good model for investigating the cellular mechanism of pathologies in the human brain (Colle et al., 2000; Head et al., 2000; Rofina et al., 2003; Skoumalova et al., 2003).

In this study we investigated the topographic distribution and morphology of PV-ir neurons in the dog claustrum. Moreover, in the light of recent anatomical studies (Baizer et al., 2014; Johnson et al., 2014) we analyzed the histological features of this enigmatic structure. Although many immunohistochemical studies have shown the presence of PV in the claustrum of different animals

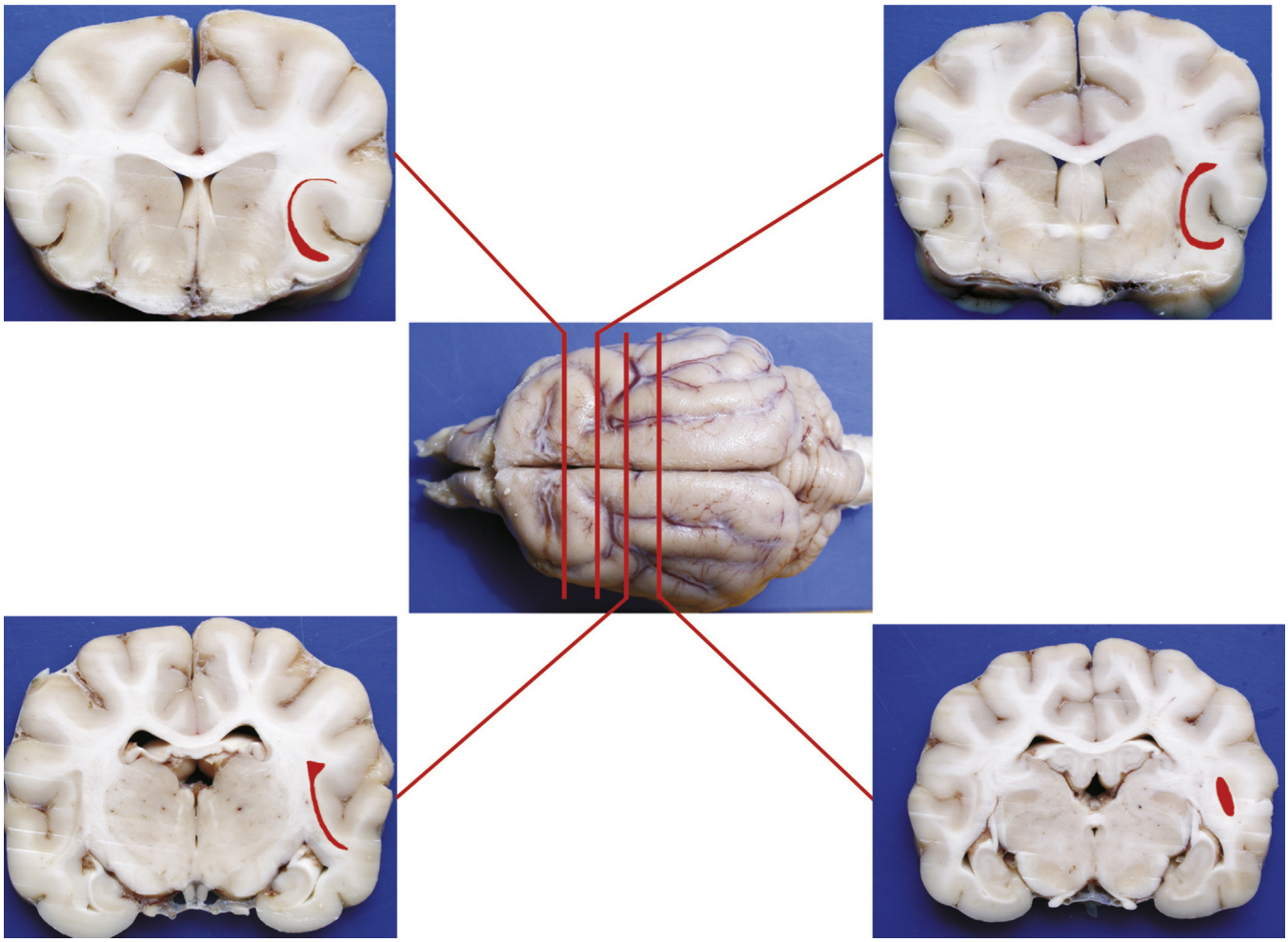

Fig. 1. Photographs of macroscopic coronal sections of the canine brain showing the four subsequent rostro-caudal levels considered for histological description and immunohistochemical characterization of PV-ir neurons. The claustrum of the right emisphere is shaded in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)