Hippocampus histological changes in epileptic dogs and cats.

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INTRODUCTION

Epilepsy-associated tissue changes can be useful for the understanding of the pathology of the disease and for therapeutic research.

Among all structures of the central nervous system (CNS), the hippocampus seems to be one of the most commonly affected areas in epilepsy. Hippocampal sclerosis (HS) is the most common pathology encountered in human Mesial Temporal Lobe epilepsy and can be described as a neuronal loss and a reactive neuroinflammation (Wagner *et al*., 2014)

OBJECTIVES

The aim of this work was to:

- assess whether the characteristics of human HS can be found in epileptic dogs and cats, mainly focusing on neuroinflammation using immunohistochemical techniques; and if so, characterize the main features of HS in dogs and cats.
- check whether neuronal loss can be evaluated by means of biomarkers.
- prove whether epileptic dogs and cats can be a useful animal model for HS.

MATERIALS AND METHODS

This study involved 11 dogs and 3 cats. Out of these animals, 8 dogs and 1 cat suffered from epilepsy, while the remaining 3 dogs and 2 cats did not show any neurological signs and counted in the control group.

Brains were sampled according to the protocol described by Matiasek *et al.* (2015) and evaluated under H/E and immunohistochemistry.

Immunohistochemical techniques used in the study.

	Antibody name	Trade house	Dilution	Pre-treatment
Neu N	Mouse anti-neuronal nuclei	Merck Millipore MAB377	1:500	Citrate buffer 0.01M pH 6, 20’ 96-98ºC water bath + 30’ room temperature
GFAP	Rabbit polyclonal Anti-Cow Glial Fibrillary Acidic Protein	Dako Z0334	1:5000	Citrate buffer 0.01M pH 6, 20’ 96-98ºC water bath + 30’ room temperature
AQP4	Polyclonal Rabbit Anti-Aquaporin 4 Antibody, CT	Millipore ab3594	1:800	Without pre-treatment
Iba-1	Goat Polyclonal to Iba-1	Abcam ab5076	1:300	Citrate buffer 0.01M pH 6, 20’ 96-98ºC water bath + 30’ room temperature

RESULTS AND DISCUSION

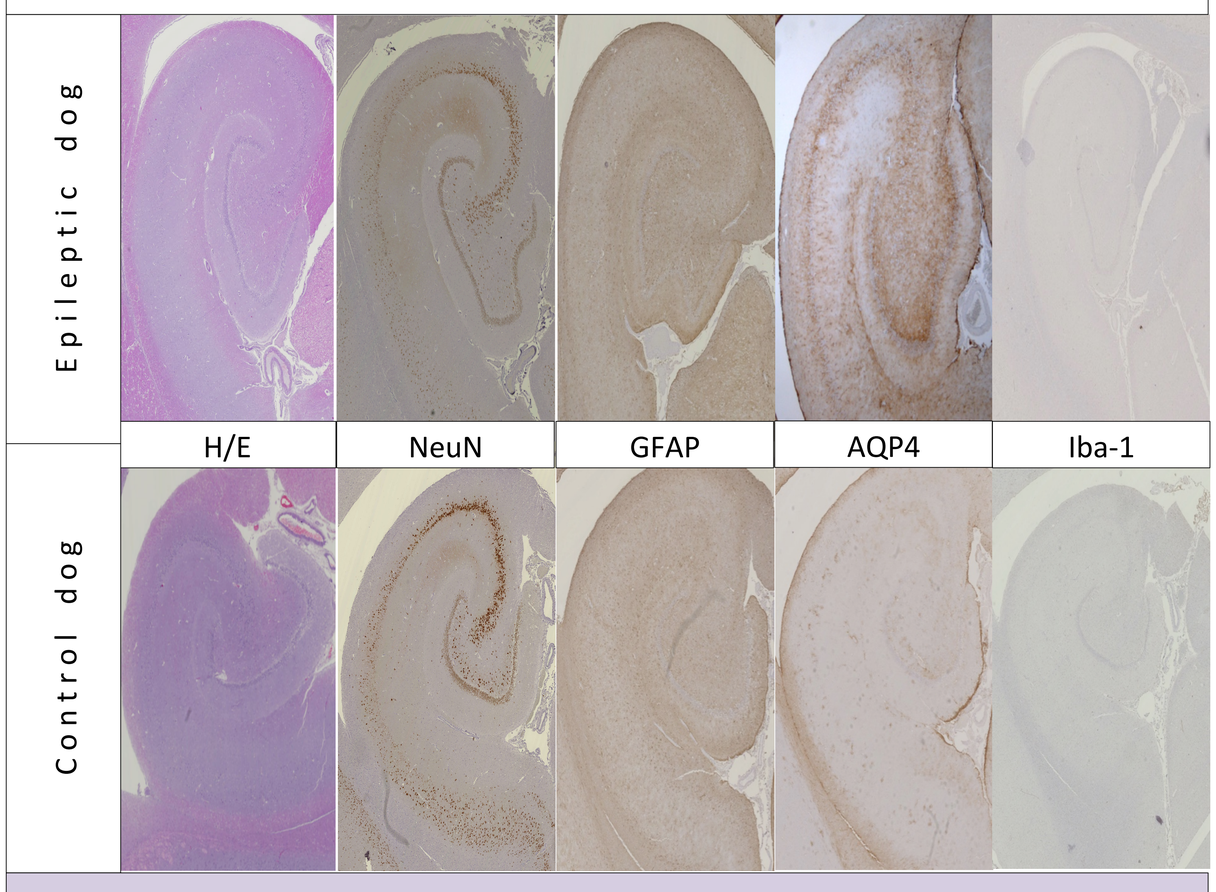

Figure 1. The comparison of all the techniques used in the study between an epileptic dog and a control one showed no important differences except for the AQP4 expression, that appeared overexpressed in the epileptic dog court. (2,5x)

Taking into account the fact that no neuronal loss was found, no neuroinflammatory alterations were expected, as neuroinflammation may occur as a consequence of neuronal loss (Fawcett and Ashe, 1999).

No other reports exist in veterinary literature about AQP4 in epileptic dog. The overexpression of AQP4 observed in epileptic dogs is similar to the one described in human revised bibliography (Coulter and Steinhäuser, 2015)

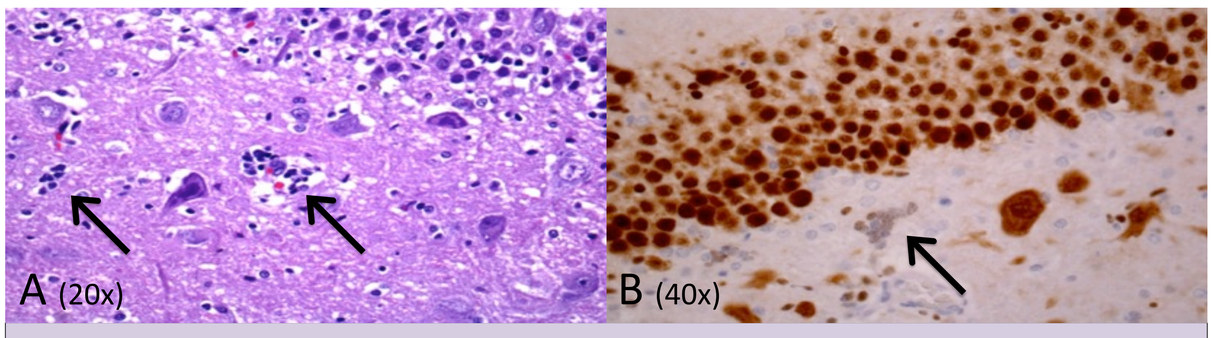

Figure 2. GD of the epileptic puppy showing cluster of apparently neurogenic cells in the polymorphic cell layer under HE (**A**) resulting immunonegative against NeuN (**B**).

Neurogenesis in DG has been widely reported in epileptic human patients but also noted in epileptic dogs (Borschensky *et al*., 2012).

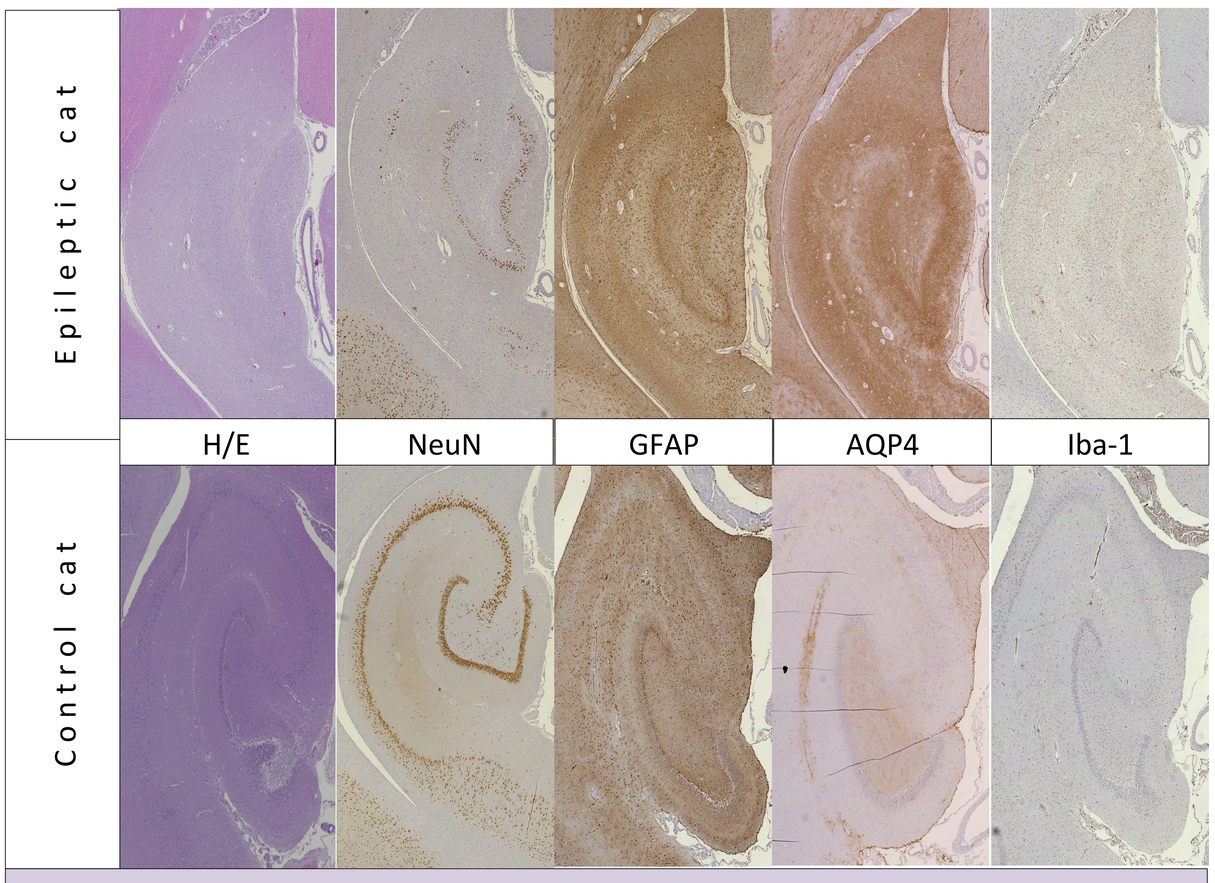

Figure 3. The comparison of all the techniques used in the study between an epileptic cat and a control one showed a clear pattern of HS with extensive neuronal loss in CA and DG and a generalized and profuse neuroinflammation. (2,5x)

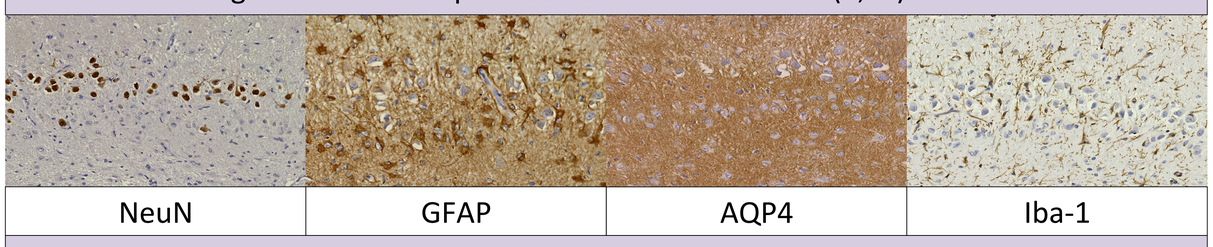

Figure 4. IHC techniques in an epileptic cat DG. The results show marker neuronal loss and granular cell dispersion, astroglial proliferation and activation and microglial proliferation. (20x)

All these results confirm HS findings (histological and immunohistochemical) in one cat with a similar pattern previously described in feline (Wagner et al, 2014) and human HS (Thom, 2014).

CONCLUSIONS

- None of the studied epileptic dogs showed histopathological and immunohistochemical changes corresponding with Hippocampal Sclerosis.
- Only the epileptic cat showed a remarkable neuronal loss and neuroinflammatory changes similar to those described for human Hippocampal Sclerosis. These findings confirm the cat as a useful animal model for epilepsy.
- AQP4 is a useful biomarker for studying epilepsy in both dogs and cats.
- A further investigation in canine epilepsy histopathogeny is needed in order to state whether dogs suffer from hippocampal sclerosis.

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