



## NOTE

Pathology

# A case of feline temporal lobe epilepsy with hippocampal sclerosis and dentate gyrus malformation

James K. CHAMBERS<sup>1)</sup>, Shinya IWASAKI<sup>1)</sup>, Shigeki IMAMOTO<sup>2)</sup>,  
Yuya NAKAMOTO<sup>3,4)</sup> and Kazuyuki UCHIDA<sup>1)\*</sup><sup>1)</sup>Laboratory of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan<sup>2)</sup>Shinjo Animal Hospital, Nara, Japan<sup>3)</sup>Neuro Vets Animal Neurology Clinic, Kyoto, Japan<sup>4)</sup>Veterinary Medical Center, Osaka Prefecture University, Osaka, Japan

**ABSTRACT.** A two-months-old, male, mixed breed cat presented with epileptic seizures. The cat was diagnosed with drug-resistant epilepsy, and died at 3-years of age. No gross lesion was found at necropsy. Histopathologically, the dentate gyrus granule cell layer of the hippocampus was irregularly arranged. Granule cells were dispersed and ectopic cells were sporadically observed in the molecular layer. The granule cells had an enlarged cytoplasm and swollen nucleus. Immunohistochemistry for NeuN and GFAP confirmed severe neuronal loss and mild gliosis in CA1. Binucleation and ischemic change were observed in the remaining pyramidal cells. This report describes a case of feline temporal lobe epilepsy and hippocampal sclerosis associated with dentate gyrus malformation.

**KEYWORDS:** dentate gyrus malformation, feline temporal lobe epilepsy, hippocampal sclerosis, histopathology, immunohistochemistry

*J. Vet. Med. Sci.*

84(5): 634–637, 2022

doi: 10.1292/jvms.22-0006

Received: 6 January 2022

Accepted: 16 March 2022

Advanced Epub:

25 March 2022

A two-months-old, male, mixed breed cat presented with recurrent epileptic seizures. Despite the administration of antiseizure drugs including phenobarbital, diazepam, levetiracetam, gabapentin, pregabalin, flunitrazepam, imepitoin and zonisamide, epileptic seizures persisted (Supplementary Table 1). Epileptic episodes started with focal seizures with facial automatism, including licking, swallowing, facial twitching, head-nodding and head-turning, and evolved into generalized tonic-clonic seizures (Supplementary Movie 1). At 6-months of age, magnetic resonance imaging revealed no significant finding in the brain (Supplementary Fig. 1A). The cat was diagnosed with drug-resistant epilepsy of unknown cause and died at 3-years and 3-months of age. Postmortem magnetic resonance imaging (Supplementary Fig. 1B) showed slight atrophy of the cerebral cortex and hippocampi compared to the previous image taken at 6-months of age (Supplementary Fig. 1A).

At necropsy, no significant changes were found in the visceral organs and the brain. The brain was fixed in 10% neutral buffered formalin and routinely embedded in paraffin for histopathological examination. Tissue sections were stained with hematoxylin and eosin (HE), and luxol fast blue and HE. Immunohistochemistry was performed using mouse monoclonal anti-NeuN antibody (clone A60, 1:100, Millipore, Temeculla, CA, USA) and rabbit polyclonal anti-glial fibrillary acidic protein (GFAP) antibody (1:400, Dako, Carpinteria, CA, USA) as previously described [4]. Immunolabeled antigens were visualized using the Dako Envision+ System (Dako). An age-matched feline brain tissue without any lesion was used for normal positive control.

Histopathological examination of the brain revealed bilateral lesions in the hippocampus (Fig. 1). No other morphological changes were observed in the brain, besides slight expansion of the cerebral sulci suggesting mild cerebral atrophy. In the hippocampus, the dentate gyrus granule cell layer was irregularly arranged in a winding line and the pyramidal cells of cornu ammonis (CA) were lost (Fig. 2). The dentate gyrus granule cells were dispersed and ectopic cells were sporadically observed in the molecular layer, occasionally showing a bi-laminated structure (Fig. 3). The granule cells had an enlarged cytoplasm and swollen nucleus. Normal hippocampus and granular layer of the same area in an age-matched cat is shown in Supplementary Fig. 2. Pyramidal cells were almost completely lost in CA1 and CA3, while pyramidal cells of CA2 and CA4 were also lost to a lesser degree (Fig. 2). Binucleation and ischemic change were observed in the remaining pyramidal cells (Fig. 4). Luxol fast blue-HE

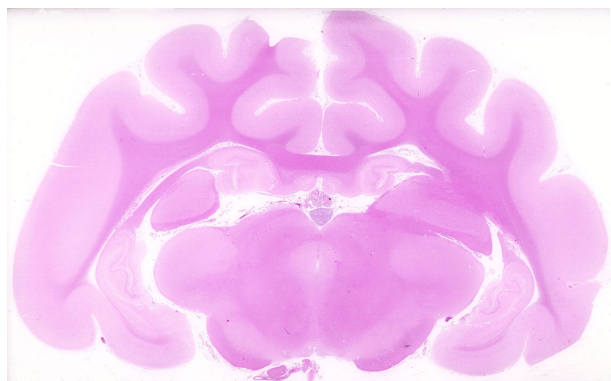
\*Correspondence to: Uchida, K.: auchidak@g.ecc.u-tokyo.ac.jp, Laboratory of Veterinary Pathology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan

(Supplementary material: refer to PMC <https://www.ncbi.nlm.nih.gov/pmc/journals/2350/>)

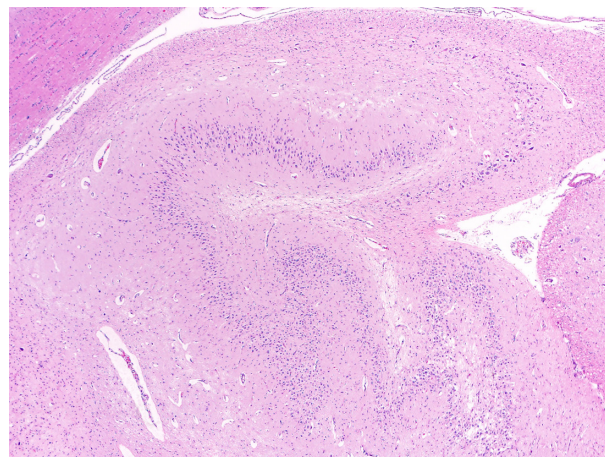
©2022 The Japanese Society of Veterinary Science



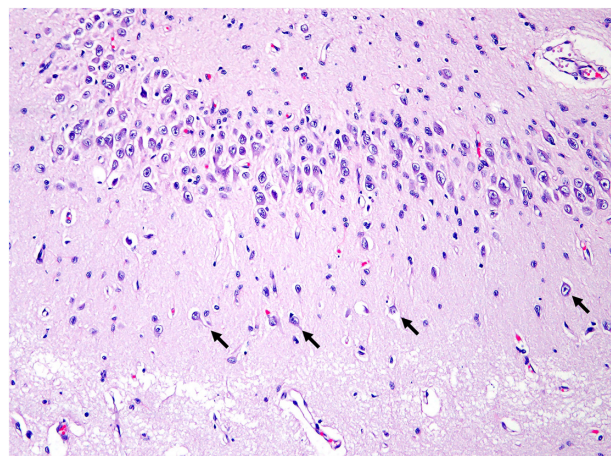
This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: <https://creativecommons.org/licenses/by-nc-nd/4.0/>)



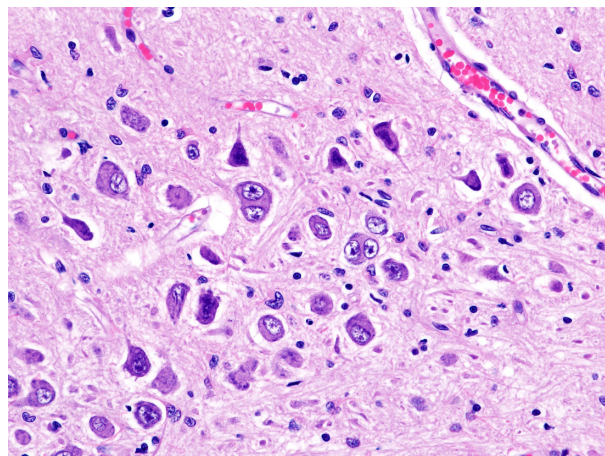
**Fig. 1.** Coronal section of the brain at the level of pineal body. The shape of both dorsal and ventral hippocampal dentate gyrus is bilaterally irregular. Cerebral sulci are mildly expanded. No significant changes are found in other areas of the brain. Hematoxylin and eosin stain; scanned image.



**Fig. 2.** Hippocampus. The dentate gyrus granule cell layer is irregularly arranged in a winding line. The pyramidal cells of cornu ammonis (CA) 1 and CA3 are lost. Hematoxylin and eosin stain;  $\times 20$ .



**Fig. 3.** Dentate gyrus. Granule cells are dispersed and have an enlarged cytoplasm with swollen nucleus. Ectopic granule cells are located in the molecular layer (arrows). Hematoxylin and eosin stain;  $\times 100$ .

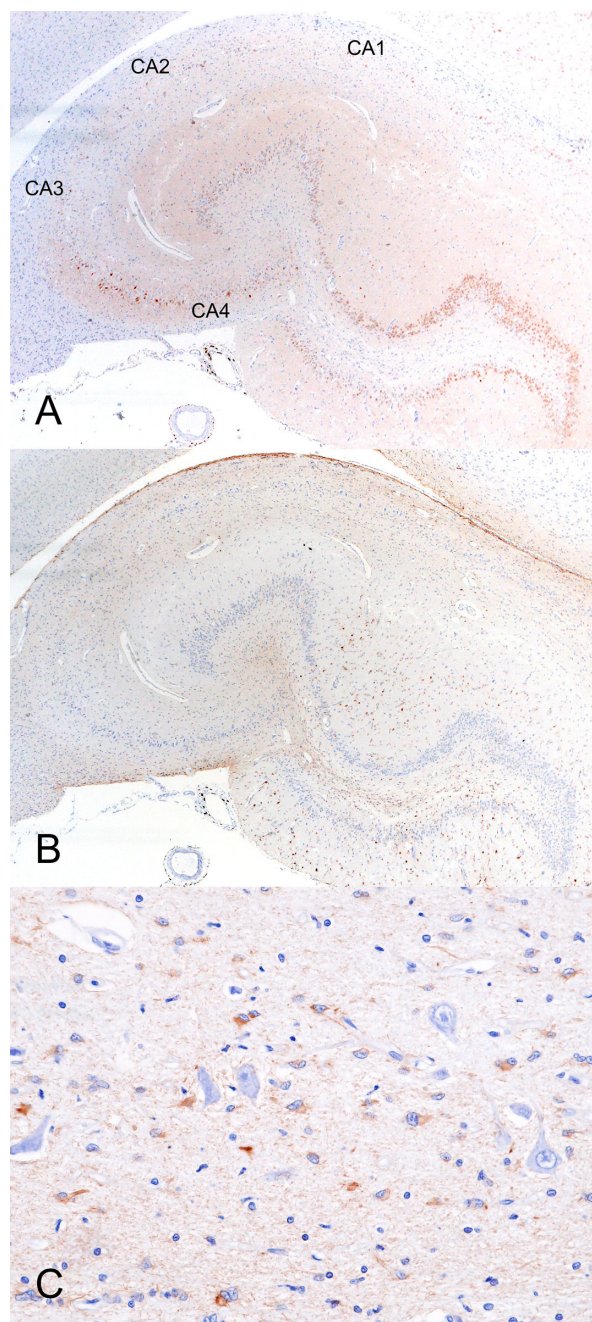


**Fig. 4.** Cornu ammonis (CA) 2–3. Remaining pyramidal cells show binucleation and ischemic change with cellular atrophy and pyknosis. Hematoxylin and eosin stain;  $\times 400$ .

staining revealed no significant change in the cerebral white matter.

A neuronal nuclear antigen NeuN has been used for evaluating aberrant distribution and loss of neuronal cells in human patients and experimental rodent models of epilepsy [2]. In the present case of feline temporal lobe epilepsy, immunohistochemistry for NeuN confirmed significant loss of pyramidal cells especially in CA1 (Fig. 5A). Pyramidal cells of CA2–4 were lost to a lesser degree. Dentate gyrus granule cell dispersion and ectopic granule cells were also depicted by immunohistochemistry for NeuN. Immunohistochemistry for GFAP revealed mild gliosis in CA1 and hilus of dentate gyrus (Fig. 5B). In CA1, where most pyramidal cells were lost, GFAP-positive astrocytes with enlarged cytoplasm were observed together with GFAP-positive astrocytic processes (Fig. 5C).

Based on the neurological signs and histopathological findings, the cat was diagnosed with feline temporal lobe epilepsy with hippocampal sclerosis and dentate gyrus malformation. Temporal lobe epilepsy in the cat has been studied mostly in experimental models for human mesial temporal lobe epilepsy, which is the most common form of focal epilepsy in humans [9, 13]. Histopathologic findings in the brains of human mesial temporal lobe epilepsy include hippocampal sclerosis and malformations, as well as neoplastic, ischemic, and inflammatory lesions. However, malformation of the hippocampus in cats with spontaneous epilepsy is rarely reported. Bilateral dentate gyrus malformation in association with hippocampal sclerosis and intraventricular meningioma was reported in a 13-year-old cat that had a history of seizures for 3 years [10]. In the present case, the cat developed



**Fig. 5.** Hippocampus. **A:** NeuN-positive neuronal cells are almost completely lost in cornu ammonis (CA) 1, and to a lesser degree in CA2–4. **B:** CA1 and the hilus of dentate gyrus are stained for glial fibrillary acidic protein (GFAP). **C:** Higher magnification of CA 1 shows GFAP-positive astrocytes with enlarged cytoplasm in areas pyramidal cells are lost. Immunohistochemistry for NeuN (A) and GFAP (B and C);  $\times 20$  (A and B);  $\times 400$  (C).

with hippocampal sclerosis [14]. Studies on seizure models of rats and human patients with epilepsy have shown increased cell proliferation of dentate granule cells [12, 15]. Granule cell enlargement and dispersion in the present case may be related to dysplasia and increased proliferation of neurons in the dentate gyrus.

No significant finding was noted on MRI at 6 months of age in the present case. A study on MRI of the hippocampus in epileptic cats revealed that cats presenting with epileptic seizures with orofacial involvement are more likely to show changes on MRI compared to epileptic cats without orofacial involvement, however mild abnormalities were difficult to detect on MRI

refractory epileptic seizures at 2 months of age and had no other complications besides hippocampal sclerosis and dentate gyrus malformation.

Hippocampal sclerosis is characterized pathologically by neuronal loss and gliosis, and further classified according to the predominantly affected area of the hippocampus [3]. In human, typical lesions show severe neuronal loss in CA1 and moderate loss in other areas excluding CA2 (type 1, classical type). In atypical cases, neuronal loss is restricted to CA1 (type 2) or CA4 (type 3). According to the International League Against Epilepsy (ILAE), immunohistochemistry for NeuN and GFAP are recommended for classifying hippocampal pathology of human mesial temporal lobe epilepsy [3]. The International Veterinary Epilepsy Task Force (IVETF) has also recommended the use of NeuN and GFAP for pathological evaluation in animals, although information on NeuN immunohistochemistry in actual animal cases of epilepsy is limited [11]. In the present case, immunohistochemistry for NeuN and GFAP confirmed severe neuronal loss and mild gliosis in CA1, which was comparable to type 1 hippocampal sclerosis in human. Pyramidal cell loss was moderate in CA3; mild in CA2 and CA4. In a retrospective study of cats with documented history of recurrent seizures and/or status epilepticus, postmortem examination revealed that CA3 was most frequently affected followed by CA4, while CA1 was spared from neuronal loss and gliosis, and thus it is considered that type 1 hippocampal sclerosis is rarely seen in cats [16]. In addition, binucleated pyramidal cells were observed in the present case, which had not been reported in previous cases of temporal lobe epilepsy with hippocampal sclerosis. Recent studies have suggested that multinucleated neurons can be formed by cell-cell fusion during development, injury and repair [7].

Granule cell pathology (GCP) in human mesial temporal lobe epilepsy has been classified into 2 types [1]: Type 1, substantial granule cell loss; Type 2, architectural abnormalities including granule cell dispersion, ectopic neurons or clusters of neurons in the molecular layer, or bi-lamination. Granule cell pathology of the present feline case was comparable to type 2 GCP. In humans, granule cell dispersion in infants with sudden unexplained death has been associated with developmental vulnerability that leads to autonomic/respiratory instability or autonomic seizures, and sleep-related death [8]. Also, mossy fiber sprouting has been associated with reparative and/or mal-adaptive event in the pathogenesis of hippocampal sclerosis. However, evaluation of mossy fiber sprouting is difficult to reproduce between laboratories, and thus the current classification of hippocampal sclerosis relies on the patterns of neuronal loss and gliosis assessed by immunohistochemistry for NeuN and GFAP, respectively [15]. In the present case, the dentate gyrus granule cells were enlarged and dispersed. Enlarged neurons are commonly seen in cortical dysplasia, the most common pathology in pediatric epilepsy patients, and are considered to be deranged radial glia, which have failed to degenerate following cortical maturation [2]. Also, enlarged granule cells with dispersion have been reported in a subset of human mesial temporal lobe epilepsy patients

[5]. Although the etiology in spontaneous cases of feline hippocampal sclerosis is yet to be elucidated, autoimmunity, ischemia, and toxins have been suggested as the primary cause. Experimental studies have shown that excessive glutamate release induces hippocampal sclerosis in cats [9]. However, it remains unclear whether the neuronal degeneration and necrosis are the cause or result of the seizure activity, or possibly both, in spontaneous cases [6]. The present case suggests that malformation of the dentate gyrus may be associated in the development of feline hippocampal sclerosis, similar to human mesial temporal lobe epilepsy.

CONFLICTS OF INTEREST. The authors declare no conflicts of interest.

ACKNOWLEDGMENT. This study was partially supported by Grant-in-Aid for Scientific Research (18H02338 and 17H01507).

## REFERENCES

- Blümcke, I., Kistner, I., Clusmann, H., Schramm, J., Becker, A. J., Elger, C. E., Bien, C. G., Merschhemke, M., Meencke, H. J., Lehmann, T., Buchfelder, M., Weigel, D., Buslei, R., Stefan, H., Pauli, E. and Hildebrandt, M. 2009. Towards a clinico-pathological classification of granule cell dispersion in human mesial temporal lobe epilepsies. *Acta Neuropathol.* **117**: 535–544. [Medline] [CrossRef]
- Blümcke, I., Thom, M., Aronica, E., Armstrong, D. D., Vinters, H. V., Palmini, A., Jacques, T. S., Avanzini, G., Barkovich, A. J., Battaglia, G., Becker, A., Cepeda, C., Cendes, F., Colombo, N., Crino, P., Cross, J. H., Delalande, O., Dubeau, F., Duncan, J., Guerrini, R., Kahane, P., Mathern, G., Najm, I., Ozkara, C., Raybaud, C., Represa, A., Roper, S. N., Salamon, N., Schulze-Bonhage, A., Tassi, L., Vezzani, A. and Spreafico, R. 2011. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc task force of the ILAE diagnostic methods commission. *Epilepsia* **52**: 158–174. [Medline] [CrossRef]
- Blümcke, I., Thom, M., Aronica, E., Armstrong, D. D., Bartolomei, F., Bernasconi, A., Bernasconi, N., Bien, C. G., Cendes, F., Coras, R., Cross, J. H., Jacques, T. S., Kahane, P., Mathern, G. W., Miyata, H., Moshé, S. L., Oz, B., Özkara, Ç., Perucca, E., Sisodiya, S., Wiebe, S. and Spreafico, R. 2013. International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: a task force report from the ILAE commission on diagnostic methods. *Epilepsia* **54**: 1315–1329. [Medline] [CrossRef]
- Chambers, J. K., Tokuda, T., Uchida, K., Ishii, R., Tatebe, H., Takahashi, E., Tomiyama, T., Une, Y. and Nakayama, H. 2015. The domestic cat as a natural animal model of Alzheimer’s disease. *Acta Neuropathol. Commun.* **3**: 78. [Medline] [CrossRef]
- Claßen, A. C., Kneissl, S., Lang, J., Tichy, A. and Pakozdy, A. 2016. Magnetic resonance features of the feline hippocampus in epileptic and non-epileptic cats: a blinded, retrospective, multi-observer study. *BMC Vet. Res.* **12**: 165. [Medline] [CrossRef]
- Fors, S., Van Meervenue, S., Jeserevics, J., Rakauskas, M. and Cizinauskas, S. 2015. Feline hippocampal and piriform lobe necrosis as a consequence of severe cluster seizures in two cats in Finland. *Acta Vet. Scand.* **57**: 41. [Medline] [CrossRef]
- Giordano-Santini, R., Linton, C. and Hilliard, M. A. 2016. Cell-cell fusion in the nervous system: Alternative mechanisms of development, injury, and repair. *Semin. Cell Dev. Biol.* **60**: 146–154. [Medline] [CrossRef]
- Kinney, H. C., Cryan, J. B., Haynes, R. L., Paterson, D. S., Haas, E. A., Mena, O. J., Minter, M., Journey, K. W., Trachtenberg, F. L., Goldstein, R. D. and Armstrong, D. D. 2015. Dentate gyrus abnormalities in sudden unexplained death in infants: morphological marker of underlying brain vulnerability. *Acta Neuropathol.* **129**: 65–80. [Medline] [CrossRef]
- Kitz, S., Thalhammer, J. G., Glantschnigg, U., Wrzosek, M., Klang, A., Halasz, P., Shouse, M. N. and Pakozdy, A. 2017. Feline temporal lobe epilepsy: review of the experimental literature. *J. Vet. Intern. Med.* **31**: 633–640. [Medline] [CrossRef]
- Klang, A., Thaller, D., Schmidt, P., Kovacs, G. G., Halasz, P. and Pakozdy, A. 2015. Bilateral dentate gyrus structural alterations in a cat associated with hippocampal sclerosis and intraventricular meningioma. *Vet. Pathol.* **52**: 1183–1186. [Medline] [CrossRef]
- Matiasek, K., Pumarola I Batlle, M., Rosati, M., Fernández-Flores, F., Fischer, A., Wagner, E., Berendt, M., Bhatti, S. F., De Risio, L., Farquhar, R. G., Long, S., Muñana, K., Patterson, E. E., Pakozdy, A., Penderis, J., Platt, S., Podell, M., Potschka, H., Rusbridge, C., Stein, V. M., Tipold, A. and Volk, H. A. 2015. International veterinary epilepsy task force recommendations for systematic sampling and processing of brains from epileptic dogs and cats. *BMC Vet. Res.* **11**: 216. [Medline] [CrossRef]
- Parent, J. M., Yu, T. W., Leibowitz, R. T., Geschwind, D. H., Sloviter, R. S. and Lowenstein, D. H. 1997. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J. Neurosci.* **17**: 3727–3738. [Medline] [CrossRef]
- Thom, M., Martinian, L., Williams, G., Stoeber, K. and Sisodiya, S. M. 2005. Cell proliferation and granule cell dispersion in human hippocampal sclerosis. *J. Neuropathol. Exp. Neurol.* **64**: 194–201. [Medline] [CrossRef]
- Thom, M., Martinian, L., Caboclo, L. O., McEvoy, A. W. and Sisodiya, S. M. 2008. Balloon cells associated with granule cell dispersion in the dentate gyrus in hippocampal sclerosis. *Acta Neuropathol.* **115**: 697–700. [Medline] [CrossRef]
- Thom, M. 2014. Review: Hippocampal sclerosis in epilepsy: a neuropathology review. *Neuropathol. Appl. Neurobiol.* **40**: 520–543. [Medline] [CrossRef]
- Wagner, E., Rosati, M., Molin, J., Foitzik, U., Wahle, A. M., Fischer, A., Matiasek, L. A., Reese, S., Flegel, T. and Matiasek, K. 2014. Hippocampal sclerosis in feline epilepsy. *Brain Pathol.* **24**: 607–619. [Medline] [CrossRef]