Brain Damage in the Epileptic Beagle Dog

D. L. Montgomery and A. C. Lee

The Collaborative Radiological Health Laboratory, College of Veterinary Medicine and Biological Sciences, Colorado State University, Fort Collins, Colo.

Abstract. Brain lesions associated with seizures in an epilepsy-prone colony of beagle dogs were studied in 68 dogs which died as a result of the disorder. Approximately 48.5% of the dogs had a relatively specific pattern of acute brain damage on microscopic examination. In all affected areas, there was a triad of lesions consisting of perineuronal and perivascular astrocytic swelling, perineuronal basophilic incrustations, and ischemic cell change in neurons. The most common areas of involvement were the cerebral cortex, basal nuclei, claustrum, amygdala, septal nuclei, dorsal thalamic nuclei, isthmus of the pyriform lobe, and hippocampus. The cerebellum was affected only rarely. In addition, intraneuronal inclusions identical to Lafora's bodies found in myoclonus epilepsy of man were detected in thalamic nuclei of six dogs.

Convulsive seizures are a relatively common clinical entity in the general canine population and may result from demonstrable organic brain disease (symptomatic epilepsy) or may occur with no evidence of any such brain abnormality (idiopathic epilepsy). In several canine breeds, there is an unusually high incidence and/or a familial predilection for idiopathic epilepsy. Despite the frequent occurrence of epilepsy in the dog, brain damage resulting from the convulsive seizures has not been recognized widely in this species. The only report dealing specifically with this subject noted only focal hemorrhage as a result of the convulsions in idiopathic epilepsy [18]. In contrast, epileptic brain damage is well recognized in man [7] and in experimentally induced convulsive seizures in laboratory animals [16].

The Collaborative Radiological Health Laboratory houses a large colony of beagle dogs for the study of the long-term effects of low-level ionizing radiation. Convulsive seizures are the second leading cause of death in dogs with an 11.9% (194/1628) incidence of observed convulsions in both control and irradiated dogs [6]. Based on clinical records, irradiation has not been shown to have a modifying effect on the incidence of seizures in this colony [6]. Since these epileptic dogs are allowed to live out their entire life-span with no specific anticonvulsive maintenance therapy, they provide a valuable resource with which to study epileptic brain damage in the canine species.

This study was conducted to define the character and neuroanatomic distribution...
of brain damage resulting from convulsive seizures and to determine if low doses of ionizing radiation would have an effect on the occurrence of these lesions.

**Materials and Methods**

A total of 68 dogs were included in this study. Twenty-one dogs were sham irradiated and each of 47 dogs received either 16 or 83 rads single-dose whole body gamma irradiation during the pre- or postnatal life up to one year of age. Except for two control dogs which were euthanatized in extremis due to status epilepticus and necropsied immediately, the dogs were found dead and necropsied after postmortem intervals ranging from less than one to 12 hours. Many of the dogs had been treated for status epilepticus the day prior to being found dead.

Thorough necropsies were done on all dogs. All brains were removed as soon as possible after death, weighed, and immersed whole in 10% neutral buffered formalin fixative. The brains were transferred to fresh formalin fixative after one day, and one and two weeks, and allowed to remain in the final fixative for an average time of three months prior to initial sectioning. Following fixation, the brains were sectioned coronally at 0.5-cm intervals. At least eight routine whole or hemicoronal sections were examined in all dogs. Sections included frontal lobes, basal nuclei, rostral and caudal thalamus, occipital cortex and mesencephalon, rostral cerebellum and brain stem at the level of the rostral or middle cerebellar peduncles, caudal cerebellum and brain stem at the level of the caudal olivary nucleus, and terminal brain stem at the level of the nucleus gracilis. Care was taken to include cross sections of the rostrodorsal and rostroventral hippocampus in order to study the relationships and relative vulnerabilities of the different parts of the hippocampal gyrus. Hematoxylin and eosin (HE)-stained sections were examined routinely. Special stains were used only to characterize specific lesions.

**Results**

The only gross abnormalities detected in the brains of dogs dying of convulsive seizures in this study were various degrees of swelling with resultant flattening of the cerebrocortical gyri and, if severe, coning of the cerebellum and compression of the caudal cerebellar folia. This was not a common lesion nor did it correlate well with the presence of microscopic lesions in these dogs.

Microscopically, in 48.5% (33/68) of the dogs there was a relatively specific pattern of acute brain damage affecting many regions of the brain. Although the appearance of the damage varied in different areas of the brain, the basic change consisted of a triad of lesions characterized by swelling of perineuronal and perivascular astrocytic processes, perineuronal basophilic incrustations, and ischemic cell change in neurons (fig. 1). Swelling of astrocytic processes was the earliest lesion detected, but since this is seen so commonly as a postmortem artifact, it was regarded as a lesion only if it was relatively well circumscribed and adjacent areas were normal, or if it was accompanied by one or both of the other lesions. In the earliest stages, swelling of perineuronal processes resulted in a scalloping effect on the neuronal cytoplasmic boundaries but as the lesion progressed the entire neuron was surrounded by a large clear vacuole fenestrated by delicate eosinophilic bands.

Perineuronal basophilic incrustations were a frequent and striking component of the lesions in convulsing dogs. The incrustations appeared at the margin of the
Fig. 1: Sommer sector of hippocampal gyrus; triad of lesions characteristic of epileptic brain damage. Ischemic cell change (open arrow), perineuronal basophilic incrustations (arrows), and swelling of perineuronal astrocytic processes. HE.

Fig. 2: Cingulate gyrus. Severe swelling of perineuronal astrocytic processes and ischemic cell change in neurons. HE.

Fig. 3: Endfolium of the hippocampal gyrus. Marked ischemic cell change in neurons and swelling of astrocytic processes. HE.
neuronal plasma membrane and were accentuated by the clear vacuoles surrounding the affected neurons. The incrustations commonly occurred in contracted, densely staining, angular neurons and were seen somewhat less frequently in neurons undergoing the final stages of ischemic cell change. The incrustations apparently persisted for a longer period than did degenerating neurons because in rare instances there was a clear vacuole containing the incrustations without a demonstrable neuron.

Neurons undergoing the ischemic cell change passed through several stages with eventual dissolution and loss of the cell. The mildest recognizable change was characterized by contraction and dense amphophilic staining of the cytoplasm or the nucleus, or both. The cytoplasm had a homogeneous granular appearance with either no apparent or only focal aggregates of Nissl substance at the periphery of the cell. As the degeneration progressed, the neuronal cytoplasm became brightly eosinophilic and more distinctly granular and the nucleus was contracted and densely basophilic. Both cytoplasmic and nuclear boundaries became somewhat indistinct. The final stage was dissolution of the cell. This began either in the nucleus or cytoplasm, but the nucleus usually was lost before the cytoplasm became indistinguishable. As a general rule, small neurons were affected most commonly although when the damage was severe, both small and large neurons had the ischemic cell change.

In addition to the changes just described, capillaries in affected areas sometimes had a prominent endothelial lining. There occasionally was a mild increase in cellularity, however there were no instances in which there was a striking glial response to the tissue degeneration.

Brain lesions associated with convulsive seizures involved specific areas throughout the brain but spared the brain stem. The distribution of lesions in both control and irradiated dogs is given in table I and, for the sake of brevity, the severity of lesions in these areas is given only for the control dogs. Although there was a slight decrease in the incidence of epileptic brain damage in the irradiated population (52.4% for control and 46.8% for irradiated dogs), this did not correlate well with the dosage of radiation or the stage of neural development when radiation was administered.

In many dogs the lesions were bilateral, but in general, the lesions affected one hemisphere more than the other. Although the data given in table I is self explanatory, there are certain brain areas that deserve additional comment. In the cerebral cortex the lesions varied in distribution but tended to involve the rostral cerebrum more severely, and in mildly affected dogs this was the only area involved. The medial aspect of the frontal lobes and the cingulate gyrus were the most common areas affected. With increasing severity the lesions extended to involve the depths of the sulci over the dorsolateral and lateral convexities of the cerebrum while in the most severely affected dogs, the lesions extended to the tips of the gyri in these areas. Within affected areas of the cerebral cortex, ischemic cell change and incrustations predominantly affected neurons in layers II and III and to a lesser extent layer V, while astrocytic swelling involved the deeper layers III through VI. In the most severely affected dogs, the astrocytic swelling had progressed to such an extent that
Table I. Distribution and severity of brain lesions in dogs with epileptic brain damage

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<tr>
<th>Dog number</th>
<th>Cerebral cortex</th>
<th>Basal ganglia</th>
<th>Claus-trum</th>
<th>Amygdala</th>
<th>Septal nuclei</th>
<th>Thalamus</th>
<th>Isthmus pyriform lobe</th>
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Total (number of dogs with brain lesions/number of epileptic dogs)

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GP = globus pallidus; C = caudate nucleus; P = putamen; - = No lesions; + = mild: lesions few and difficult to find; ++ = moderate: lesions easily discernible at moderate magnification; +++ = severe: lesions detectable at low magnification.
the affected area had an intensely vacuolated appearance (fig. 2). In two dogs, which were not included in the table of epileptic brain damage, the only brain lesions were in the cerebral cortex at the boundary zone between the middle and rostral cerebral arteries but in these dogs, astrocytic swelling was not a prominent feature.

In the basal nuclei the globus pallidus was involved most frequently and unlike other brain areas, the damage tended to be bilaterally equal in severity. The lesions in this nucleus were located in the rostromedial aspects while the caudal and lateral areas were unaffected. In the caudate nucleus the dorsolateral aspects of the head and body were involved while the ventromedial aspects of these areas and the tail of the nucleus were normal. In the only dog with putaminal involvement, the lesion was mild and consisted of only a scattering of ischemic neurons. Although the claustrum and amygdala often are considered parts of the basal nuclei, they were represented separately because lesions in the claustrum were associated with damage to the olfactory lobe and lesions in the amygdala were associated with lesions in the hippocampal formation. In the least affected dogs, the claustrum adjacent to the external capsule was the only area involved. As the severity increased, the lesions extended into the olfactory lobe but tended to spare the pyramidal cell layer in this lobe. In the amygdala, the cortical and basal areas tended to be affected only slightly more frequently than the lateral, ventral, rostral, and central areas.

Several nuclei were affected in the thalamus. The dorsomedial and rostrolateral nuclei were most frequently affected with only a few dogs having lesions in the lateral caudal and medial geniculate nuclei.

The dorsal horn of the hippocampus was involved more frequently than the ventral horn and in dogs where both the dorsal and ventral horns were affected, the lesions tended to be more severe in the dorsal horn. Within the hippocampal gyrus, the Sommer sector (H₁) and endfolium (H₂-H₅) (fig. 3) were affected most frequently while the H₂ region was affected only in the most severely affected dogs, and the dentate gyrus never was involved. The indusium griseum, which is regarded as a rudimentary part of the hippocampal formation in higher animals, was affected in those dogs having lesions in the hippocampal gyrus.

No lesions were common in the cerebellar cortex in the dogs in this study, and when present, they were characterized by ischemic cell change involving Purkinje's cells at the base and sides of the folia. No vacuolization nor incrustations were present.

One additional lesion was detected in the brains of epileptic dogs. In six dogs, only one of which had epileptic brain damage as described above, Lafora-like inclusion bodies having identical staining characteristics to Lafora's bodies seen in myoclonus epilepsy of man were found in neurons of the dorsomedial, rostrolateral, lateral geniculate, and lateral caudal thalamic nuclei. The bodies ranged in size from 10 to 25 μm and, when the nucleus was apparent, it was pushed to an eccentric position within the cell. With hematoxylin and eosin staining the bodies had a dense basophilic central core surrounded by a pale, poorly stained zone containing fine radiating
basophilic fibrils (fig. 4a). The central core of the bodies stained strongly periodic acid-Schiff (PAS) positive (fig. 4b) and black with Weil's stain (fig. 4c).

Discussion

The results of this study indicate that an acute and selective pattern of brain damage can occur as a consequence of convulsive seizures in the dog. It is of interest that many dogs included in this study were in status epilepticus prior to being found dead, and it is probable that the lesions described here resulted from the intense, prolonged convulsive seizures characteristic of this state. In support of this, status epilepticus in man [7] and experimentally induced in laboratory animals [2, 13, 15−17, 19] results in acute brain damage almost identical in character and distribution to the lesions described in this study. In addition, there is a close correlation between the lesions in these dogs and those described in many types of hypoxic and/or hypoglycemic brain injury [4].

There are four points concerning the character of epileptic brain damage which need to be emphasized. The first of these concerns the perineuronal and perivascular vacuolization. As indicated in the results, these vacuoles have been shown to represent the swollen processes of astrocytes [16, 19]. This is a common postmortem artifact however, and its occurrence should be interpreted with caution. In the present study, such a change occurred in areas of selective vulnerability to epileptic brain damage and commonly was accompanied by the ischemic cell change and incrustations. When this lesion is well localized, it is recognized easily at low magnification and can be used as a marker for closer examination, thus facilitating the detection of the other lesions, especially when the brain is fixed well. The second point concerns the postmortem artifact of 'dark' neurons. These are seen readily when the animal brain is removed and immersed directly into formalin fixative and is regarded as an artifact due to trauma [5]. The 'dark' neuron appears contracted, angular, and stains deeply with routine histological stains [5]. This artifact can and has been interpreted as an
early stage of ischemic cell change but, at least in the cerebral and cerebellar cortices, the distribution of affected cells may aid in the distinction between the two changes. In these areas, 'dark' neurons are most numerous at the tips of the cerebrocortical gyri and cerebellar folia while ischemic cell change is most common in the depths of the sulci [5]. Third, although the term ischemic cell change implies a vascular abnormality, it should be remembered that it may occur in all types of hypoxia and hypoglycemia [4, 5], despite the fact that hypoxic and hypoglycemic brain damage are reported to have a different pathogenesis [1]. Last, there was little evidence of a pronounced glio-mesodermal reaction associated with the epileptic brain damage in the dog in contrast to reports in man [7]. This may suggest that the dogs died prior to the onset of the change or before the change could become prominent.

The neuroanatomic distribution of epileptic brain damage was consistent and relatively widespread in the dogs examined in this study. The precise interactions rendering these areas of the brain vulnerable to epileptic brain damage in both man and animals is not known. In only two dogs was there a distribution of lesions indicating a definite boundary-zone pattern and in these dogs, the lesions were in the dorsolateral cerebral cortex at the junction of the rostral and middle cerebral arteries. This boundary-zone distribution of lesions suggests a decreased perfusion of the entire brain (oligemic hypoxia) [4] and it is of interest that one of these dogs also had myocardial degeneration, suggesting a cause-effect relationship. Although a boundary-zone effect may be seen in all types of hypoxia-ischemia, it does not account for all the lesions described here or in previous studies [4].

Just as there is no explanation for the distribution of epileptic brain damage, there is also no simple explanation for the pathogenesis of the lesions. It is known that a multiplicity of systemic and local abnormalities such as pyrexia, arterial hypotension, hypoxia, acidosis, and hypoglycemia may accompany convulsive seizures. When these factors were controlled strictly to determine which played a role in the genesis of epileptic brain damage, there was no clear evidence incriminating any one factor except that control of pyrexia and arterial hypotension abolished the cerebellar damage [2, 15, 17]. Since cerebellar lesions were not a common occurrence in the dogs of this study, it may indicate that the brain damage in these dogs was not related to these two factors. In another study of cerebral energy metabolism during status epilepticus, it was concluded that the brain possesses only a limited capacity to adjust its metabolism to meet the energy demands during prolonged convulsive seizures and this capacity would eventually fail unless the electrical discharges themselves were brought under control [9]. Other studies have indicated that failure of cerebral energy metabolism may not play a major role in the production of epileptic brain damage [2, 10]. In light of these studies, it is believed that epileptic brain damage is possibly the result of the greatly enhanced neuronal activity accompanied by the altered metabolic state [10].

This documentation of epileptic brain damage in the canine species should aid in the postmortem evaluation of dogs with convulsive seizures. Several reports describ-
ing polioencephalomalacia in the dog have indicated similar lesions in many of the brain areas affected in dogs of this study [3, 11, 14]. In these reports, many of the dogs had a clinical history of convulsive seizures. In two reports, an association was made between the convulsive seizures and the brain damage [3, 14].

Regarding the potential effects of radiation exposure on the incidence of epileptic brain damage in these dogs, it would seem that irradiation did not significantly alter the incidence or character of such damage. Before drawing definite conclusions however, these findings must be correlated with clinical data which should include the age of onset, sex, number of observed seizures and the relative severity of these convulsive episodes, and the age at the time of death. Since this report deals primarily with the character of epileptic brain damage in the dog, these clinical variables were beyond the scope of this study.

One further point of discussion needs to be made concerning the occurrence of Lafora's bodies in the brain of dogs. Lafora's bodies have been reported in thalamic neurons of epileptic beagle dogs [12], and in the cerebellum of epileptic dogs [8]. The finding of only six dogs with Lafora's bodies out of 68 dogs in the present study does not indicate a close association between these bodies and epilepsy, at least in the Collaborative Radiological Health Laboratory beagle dogs. In light of this, caution should be exercised in drawing too close an association between epilepsy in the beagle dog and myoclonus epilepsy (Lafora's disease) of man.

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References


Request reprints from Dr. D. L. Montgomery, P. O. Box 3200, Amarillo, Texas 79106 (USA).