Quantitative analysis of brain perfusion parameters in dogs with idiopathic epilepsy by use of magnetic resonance imaging

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OBJECTIVE
To quantitatively analyze brain perfusion parameters in dogs with idiopathic epilepsy (IE) by use of MRI and to compare those findings with brain perfusion parameters for healthy dogs.

ANIMALS
12 client-owned dogs with IE.

PROCEDURES
For each dog, standard MRI and perfusion-weighted imaging (before and after injection of gadoteric acid contrast medium) sequences of the brain were obtained during the interictal period by means of the same protocol used in a comparable study of healthy dogs. Time of contrast medium arrival, time to peak contrast enhancement, mean contrast transit time, and cerebral blood flow were calculated for the caudate nucleus, thalamus, piriform lobe, hippocampus, semioval center, and temporal cerebral cortex. Parameters for each structure were compared between dogs with IE and healthy dogs.

RESULTS
Dogs with IE had a significantly greater mean time of contrast arrival and lower mean cerebral blood flow than healthy dogs. Differences in cerebral blood flow between dogs with IE and healthy dogs were most pronounced in the piriform lobe, thalamus, and temporal cerebral cortex. The mean contrast transit time did not differ between dogs with IE and healthy dogs.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested that, compared with healthy dogs, dogs with IE have decreased blood perfusion of the brain. Findings of this study can be used as a basis for further research into functional changes within the brains of epileptic dogs during the interictal phase. (Am J Vet Res 2018;79:433–442)

Epilepsy is a brain disorder characterized by repeated seizure activity over a long period of time.1 Epilepsy has been reported in many breeds of dogs throughout the world.1,3 Idiopathic epilepsy is defined as recurrent seizures with no structural or reactive changes in the brain of patients with a genetic predisposition for epilepsy.1 It is one of the most common neurologic disorders of dogs and accounts for predisposition for epilepsy.

The age of onset for IE is usually between 1 and 5 years, but IE has been diagnosed in dogs ranging in age from 6 months to 10 years.1,7 During interictal periods, dogs with IE generally appear clinically normal, and standard MRI of the brain and CBC, serum biochemical analysis, serum bile acid concentration, and CSF analysis results are unremarkable.1 Dogs with epilepsy require lifelong treatment with antiepileptic drugs, and up to 30% of those dogs can develop pharmacoresistance to antiepileptic drugs, or refractory epilepsy.1,8

In human patients with pharmacologically refractory seizures, invasive treatments are available when an epileptogenic focus is identified. Surgical resection of the area of the brain that triggers seizures (eg, partial or total temporal lobe resection) is a common technique.9–11 In patients where the epileptogenic area is located in an area of the brain that cannot be safely removed (eg, areas of speech, memory, or motor or sensory function), multiple subpial transections are an option.12 Deep brain stimulation is a fairly new and promising technique for the treatment of refractory epilepsy in human patients that has been associated with a reduction in the number of seizures.15–16 All invasive procedures require exact localization of the epileptogenic zone. Standard

ABBREVIATIONS
Δ Mean difference
AIF Arterial input function
CBF Cerebral blood flow
df Degrees of freedom
IE Idiopathic epilepsy
MTT Mean transit time
PWI Perfusion-weighted imaging
ROI Region of interest
T0 Time of arrival
TTP Time to peak

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MRI can be used to detect morphological changes in the brain of epileptic patients; however, morphological changes are not present in the brain of patients with IE. Functional MRI is a collection of various techniques such as PWI that facilitate visualization of physiologic processes, and those techniques are useful in patients prior to invasive treatments.

In human patients with epilepsy, PWI findings vary depending on the cause of epilepsy. In patients with temporal lobe epilepsy, the ictal focus frequently has abnormally increased perfusion during ictus and is hypoperfused during the interictal period. Investigators of another study of epileptic patients report relative hyperperfusion in the hippocampus and hypoperfusion of the parahippocampal gyrus on the ictogenic side during the interictal period, with normalization of perfusion during the postictal period following a single seizure.

Information about brain perfusion in dogs with epilepsy is scarce. To our knowledge, only 1 study has been published that describes the brain perfusion of dogs. In that study, single-photon emission CT was used to characterize brain perfusion in 10 dogs implanted with a vagal nerve stimulation system to model epilepsy in humans, and results indicated that blood perfusion in the subcortical area was decreased during the interictal period. The aim of the study reported here was to quantitatively analyze brain perfusion in dogs with IE. We wanted to assess the feasibility of the use of PWI to detect areas with altered perfusion, which could be related to seizure activity, during the interictal period, and compare those findings with brain perfusion parameters determined for healthy dogs (controls) of a comparable study.

Materials and Methods

Animals

The study had a prospective design. All investigations were conducted in compliance with the German Animal Protection Law. All dogs were client owned and resided with their owners. The owners of all dogs provided consent for their pets to be evaluated in the study. Approval from the Committee on the Ethics of Animal Experiments of the Justus-Liebig-University and local Hessian government was not required because all examinations were performed for diagnostic reasons with the owners’ consent. The MRI protocol used was the same as that for healthy dogs of a previous study conducted by our research group, which was approved by the Committee on the Ethics of Animal Experiments of the Justus-Liebig-University Giessen and local Hessian government (reference number: V54-19c2015(1)GI18/17 Nr. 78/2011).

From January 2013 to January 2014, 89 dogs with a history of seizures were examined by the neurology unit at the Justus-Liebig-University of Giessen. For each dog, a physical and complete neurologic examination were performed by a board-certified veterinary neurologist, as were a CBC, serum biochemical profile, CSF analysis, and determination of serum ammonia and preprandial and postprandial serum bile acid concentrations.

Only dogs with IE were included in the study. For the purpose of this study, IE was defined as recurrent generalized tonic-clonic seizures during the 4 weeks prior to examination. Generalized tonic-clonic seizures were defined as bilaterally symmetric tonic-clonic limb movements accompanied by autonomic clinical signs (eg, salivation and urination) and an impaired state of consciousness. Seizures were classified on the basis of video analysis. Dogs that had only 1 seizure during the 4 weeks prior to examination and dogs with partial seizures or complex partial seizures were excluded from the study. Additionally, dogs included in the study had to be between 6 months and 3 years old at the time of seizure onset. All dogs were examined interictally, and results of the physical and neurologic examinations and all laboratory and diagnostic test results including CSF analysis and standard MRI of the brain had to be unremarkable. Standard MRI images were assessed visually. Dogs with a history of dystocia or head trauma and dogs receiving antiepileptic drugs were also excluded from the study.

MRI protocol

Each dog was anesthetized for collection of CSF for analysis and MRI. The anesthetic and MRI examination protocols used were the same as those used for the healthy dogs of another study. The MRI examinations were performed by use of a 1.0-T superconductive system and sensitivity-encoding coil. For each dog, dorsal and transverse T2-weighted, transverse T2-weighted fluid-attenuated inversion recovery, and transverse T2*-weighted gradient echo sequences were acquired in addition to transverse T1-weighted and dorsal T1-weighted gradient echo sequences before and after contrast medium administration. Perfusion-weighted images were acquired in the dorsal plane with a dynamic multishot fast-field echo–echo planar imaging sequence as described. Slices were oriented parallel to the base of the skull and had a thickness of 5.0 mm. One slice was acquired through the thickest part of the caudate nucleus. For each slice, 40 dynamics were acquired at 1.6-millisecond intervals. At the 10th dynamic, gadoteric acid contrast medium (0.2 mmol/kg, IV) was injected with a double-headed injection pump at a rate of 5 mL/s followed by 20 mL of isotonic Ringer solution as described.

Perfusion analysis

Analysis of perfusion data was identical to that described for healthy dogs and involved deconvolution techniques and variate fitting by use of an AIF at a dedicated workstation. Regions of interest were manually drawn around the caudate nucleus, thalamus, piriform lobe, hippocampus, semioval center, and temporal cerebral cortex lateral to the semioval center. The size and shape of the ROIs were not standardized but rather adapted to each brain; however,
owing to the inherent shape of most brain structures, the shape of the ROIs varied only slightly. Each ROI was drawn on 1 representative slice; because the slice thickness was 5.0 mm, most ROIs were visible on only 1 slice. All ROIs were drawn by the same investigator (CV) and were repeated 5 times with 1 to 2 weeks between each drawing; the process of repeated ROI drawings is henceforward referred to as the ROI selection run. Integrated software was used to calculate the number of seconds between contrast medium injection and arrival at an ROI (T0), number of seconds between contrast medium injection and maximum concentration in an ROI (TTP), mean number of seconds required for the contrast medium to pass through the ROI (MTT), volume of blood in an ROI divided by the mass of the ROI (cerebral blood volume), and blood flow through an ROI divided by the mass of the ROI (CBF; measured in mL/100 g/min) as described.26,27 The mathematical model applied to the data was the same as that described for healthy dogs.25 Cerebral blood volume was not assessed further because the cerebral blood volume within an ROI is equivalent to the product of CBF and MTT.28

Statistical analysis

The statistical methods used for this study were similar to those described in a comparable study25 of healthy dogs. A correction was not applied to the data because it might have produced additional correlations owing to the small and heterogeneous group of diseased dogs that was evaluated.

The Pearson correlation coefficient (r) was used to determine whether there was a linear relationship between T0 and TTP. The Spearman correlation coefficient (p) was used to assess the proposed nonlinear relationships between CBF and T0 and between CBF and MTT. A nonparametric test was chosen for those assessments because the exact nature of the nonlinear relationships between those parameters was unknown. Also, the nonlinear relationship might have affected the distributions of the parameters, which would invalidate the normal distribution required for a parametric test. Finally, although the mathematical model used did predict how the distributions for the parameters might look, we wanted to use a test that could independently verify the relationships.

Dependence of brain perfusion parameters (T0, CBF, and MTT) on anatomic or user-dependent effects was evaluated by fitting several mixed linear models with varying fixed and random terms. To test the significance of a given variable (AIF, ROI [anatomic region], ROI selection run, and hemisphere [right or left]), 2 models were fitted: 1 that included the given variable and another that did not include that variable. To determine the significance of factors entered into a model, the mixed linear models were tested against each other by use of a χ² test. The models were built by the inclusion of possible interactions between fixed effects. Random effects were included in all models to account for repeated measures within ROIs and dogs. The MTT was modeled by use of a normal distribution, whereas T0 and CBF were modeled by use of γ-distributions in a manner analogous to the models described for healthy dogs.25 A log-link function was used to model all γ-distributed variables. For each final model, the regression coefficients for fixed effects were reported along with their accompanying 95% confidence intervals, which were estimated with the Wald method. Although γ-distributions are specified by rate and shape, results for all perfusion parameters were reported as the mean ± SD, which also uniquely specifies the distribution, to allow for comparison with published values. All mixed linear models were built with dedicated software packages,29 and only variables with values of P ≤ 0.01 were retained in the final models. Results of Q-Q plots revealed that the distribution assumptions modeled by the mathematical models for dogs with IE were not as precise as those for healthy dogs.25 (Supplementary Figure S1, available at www.avmajournals.avma.org/doi/suppl/10.2460/ajvr.79.4.433). However, the same types of distributions were used for this study so that the data from the healthy dogs of that other study25 could be incorporated into the mixed linear model analyses and compared with data for the dogs with IE.

As in the study25 involving healthy dogs, an additional cluster analysis was performed for CBF, in which the data were separated into 2 clusters on the basis of the number of arterial inlets included in the ROI. Unless otherwise specified, all statistical analyses were performed with commercially available software,30 and values of P ≤ 0.01 were considered significant.

Results

Twelve dogs met the inclusion criteria and were enrolled in the study. The study population included 2 mixed-breed dogs, 2 Labrador Retrievers, and 1 each Airedale Terrier, American Staffordshire Terrier, Australian Shepherd, Border Collie, French Bulldog, German Wirehaired Pointer, Giant Schnauzer, and Landseer. There were 5 sexually intact females, 4 neutered males, and 3 sexually intact males that had a median age of 2 years (24 months; range, 6 to 108 months) and body weight of 27.7 kg (range, 15.0 to 47.0 kg) at the time of examination. The median age at the first observed seizure was 24 months (range, 6 to 36 months), and the duration since the last seizure at the time of the examination ranged from 1 to 9 days.

A significant (P < 0.001) strong negative correlation (r = −0.92) was identified between T0 and TTP. There was also a significant negative correlation between T0 and CBF (r = −0.57; P < 0.001) and between MTT and CBF (r = −0.20; P < 0.001). We chose to not evaluate TTP any further because of its almost perfect, albeit negative, linear correlation with T0.

The final mixed linear models for T0 (Table 1), CBF (Table 2), and MTT (Table 3) were summarized. Time of arrival was significantly (P < 0.001) associated with disease status (χ² [df: 1] = 43.228), and the mean
For each dog, PWIs were acquired in the dorsal plane with a dynamic multishot fast-field echo–echo planar imaging sequence as described.23 Slices were oriented parallel to the base of the skull and had a thickness of 5.0 mm. One slice was acquired through the thickest part of the caudate nucleus. For each slice, 40 dynamics were acquired at 1.6-millisecond intervals. At the 10th dynamic, gadoteric acid contrast medium (0.2 mmol/kg, IV) was injected with a double-headed injection pump at a rate of 5 mL/s followed by 20 mL of isotonic Ringer solution. Regions of interest were manually drawn around the caudate nucleus, thalamus, piriform lobe, hippocampus, semioval center, and temporal cerebral cortex lateral to the semioval center. The size and shape of the ROIs were not standardized but rather adapted to each brain; however, owing to the inherent shape of most brain structures, the shape of the ROIs varied only slightly. Each ROI was drawn on 1 representative slice; because the slice thickness was 5.0 mm, most ROIs were visible on only 1 slice. All ROIs were drawn by the same investigator (CV) and were repeated 5 times with 1 to 2 weeks between each drawing (ie, ROI selection run). Integrated software was used to calculate the number of seconds between contrast medium injection and arrival at an ROI (T0), mean number of seconds required for the contrast medium to pass through the ROI (MTT), and blood flow through the ROI divided by the mass of the ROI (CBF). Dependence of brain perfusion parameters (T0, CBF, and MTT) on anatomic (AIF, ROI, cerebral hemisphere [right or left], and dog health status [IE or healthy]) or user-dependent effects (ROI selection run) was evaluated by fitting several mixed linear models with various fixed and random terms. Models were built with the inclusion of possible interactions between fixed effects. Random effects were included in all models to account for repeated measures within ROIs and dogs. The MTT was modeled by use of a normal distribution, whereas T0 and CBF were modeled by use of γ-distributions. A log-link function was used to model all γ-distributed variables. For each final model, the regression coefficients for fixed effects were reported along with their accompanying 95% confidence intervals, which were estimated with the Wald method. Only variables with values of $\hat{P} \leq 0.01$ were retained in the final models.

### Table 1—Final mixed linear model for T0 as determined by MRI for 6 ROIs (semioval center, temporal cerebral cortex, hippocampus, piriform lobe, caudate nucleus, and thalamus) of the brain for 12 dogs of various breeds with IE and 8 healthy adult Beagles of another study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fixed-effect coefficient</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>33.28</td>
<td>33.02 to 33.53</td>
</tr>
<tr>
<td>Healthy</td>
<td>–9.14</td>
<td>–9.54 to –8.73</td>
</tr>
</tbody>
</table>

For each dog, PWIs were acquired in the dorsal plane with a dynamic multishot fast-field echo–echo planar imaging sequence as described.23 Slices were oriented parallel to the base of the skull and had a thickness of 5.0 mm. One slice was acquired through the thickest part of the caudate nucleus. For each slice, 40 dynamics were acquired at 1.6-millisecond intervals. At the 10th dynamic, gadoteric acid contrast medium (0.2 mmol/kg, IV) was injected with a double-headed injection pump at a rate of 5 mL/s followed by 20 mL of isotonic Ringer solution. Regions of interest were manually drawn around the caudate nucleus, thalamus, piriform lobe, hippocampus, semioval center, and temporal cerebral cortex lateral to the semioval center. The size and shape of the ROIs were not standardized but rather adapted to each brain; however, owing to the inherent shape of most brain structures, the shape of the ROIs varied only slightly. Each ROI was drawn on 1 representative slice; because the slice thickness was 5.0 mm, most ROIs were visible on only 1 slice. All ROIs were drawn by the same investigator (CV) and were repeated 5 times with 1 to 2 weeks between each drawing (ie, ROI selection run). Integrated software was used to calculate the number of seconds between contrast medium injection and arrival at an ROI (T0), mean number of seconds required for the contrast medium to pass through the ROI (MTT), and blood flow through the ROI divided by the mass of the ROI (CBF). Dependence of brain perfusion parameters (T0, CBF, and MTT) on anatomic (AIF, ROI, cerebral hemisphere [right or left], and dog health status [IE or healthy]) or user-dependent effects (ROI selection run) was evaluated by fitting several mixed linear models with various fixed and random terms. Models were built with the inclusion of possible interactions between fixed effects. Random effects were included in all models to account for repeated measures within ROIs and dogs. The MTT was modeled by use of a normal distribution, whereas T0 and CBF were modeled by use of γ-distributions. A log-link function was used to model all γ-distributed variables. For each final model, the regression coefficients for fixed effects were reported along with their accompanying 95% confidence intervals, which were estimated with the Wald method. Only variables with values of $\hat{P} \leq 0.01$ were retained in the final models.

### Table 2—Final mixed linear model for CBF as determined by MRI for 6 ROIs (semioval center, temporal cerebral cortex, hippocampus, piriform lobe, caudate nucleus, and thalamus) of the brain for the dogs of Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fixed-effect coefficient</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semioval center</td>
<td>0.44</td>
<td>0.26 to 0.62</td>
</tr>
<tr>
<td>Temporal cerebral cortex</td>
<td>0.42</td>
<td>0.16 to 0.68</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.68</td>
<td>0.39 to 0.97</td>
</tr>
<tr>
<td>Piriform lobe</td>
<td>0.20</td>
<td>–0.63 to 1.04</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>0.34</td>
<td>–0.10 to 0.79</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.20</td>
<td>–0.11 to 0.50</td>
</tr>
<tr>
<td>Semioval center, cluster</td>
<td>0.13</td>
<td>–0.09 to 0.34</td>
</tr>
<tr>
<td>Temporal cerebral cortex, cluster</td>
<td>0.47</td>
<td>–0.07 to 1.01</td>
</tr>
<tr>
<td>Hippocampus, cluster</td>
<td>0.43</td>
<td>–0.10 to 0.97</td>
</tr>
<tr>
<td>Piriform lobe, cluster</td>
<td>0.51</td>
<td>–0.32 to 1.35</td>
</tr>
<tr>
<td>Caudate nucleus, cluster</td>
<td>0.56</td>
<td>–0.46 to 1.59</td>
</tr>
<tr>
<td>Thalamus, cluster</td>
<td>0.38</td>
<td>–0.08 to 0.84</td>
</tr>
<tr>
<td>Semioval center, healthy</td>
<td>0.36</td>
<td>0.19 to 0.52</td>
</tr>
<tr>
<td>Temporal cerebral cortex, healthy</td>
<td>0.34</td>
<td>0.11 to 0.57</td>
</tr>
<tr>
<td>Hippocampus, healthy</td>
<td>–0.01</td>
<td>–0.31 to 0.29</td>
</tr>
<tr>
<td>Piriform lobe, healthy</td>
<td>0.85</td>
<td>0.12 to 1.58</td>
</tr>
<tr>
<td>Caudate nucleus, healthy</td>
<td>–0.06</td>
<td>–0.79 to 0.67</td>
</tr>
<tr>
<td>Thalamus, healthy</td>
<td>0.65</td>
<td>0.40 to 0.90</td>
</tr>
</tbody>
</table>

### Table 3—Final mixed linear model for MTT as determined by MRI for 6 ROIs (semioval center, temporal cerebral cortex, hippocampus, piriform lobe, caudate nucleus, and thalamus) of the brain for the dogs of Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fixed-effect coefficient</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semioval center</td>
<td>3.79</td>
<td>3.43 to 4.15</td>
</tr>
<tr>
<td>Temporal cerebral cortex</td>
<td>–0.33</td>
<td>–0.68 to 0.02</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.29</td>
<td>–0.054 to 0.64</td>
</tr>
<tr>
<td>Piriform lobe</td>
<td>–0.06</td>
<td>–0.41 to 0.28</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>–0.44</td>
<td>–0.80 to –0.10</td>
</tr>
<tr>
<td>Thalamus</td>
<td>–0.35</td>
<td>–0.70 to 0.00</td>
</tr>
</tbody>
</table>

See Table 1 for key.

An additional cluster analysis was performed for CBF, in which the data were separated into 2 clusters on the basis of the number of arterial inlets included in the ROI. See Table 1 for remainder of key.

T0 for dogs with IE was greater than that for healthy dogs, regardless of the anatomic location in the brain (ROI). Cerebral blood flow was also significantly ($P < 0.01$) associated with disease status ($\chi^2 [df, 6] = 21.53$), and the mean CBF for dogs with IE was lower than that for healthy dogs. When the cluster analysis results were included in the mixed linear model for CBF, the fixed effect for disease status ($\chi^2 [df, 6] = 30.07$) was still significant ($P < 0.01$). The magnitude of the difference in CBF between healthy dogs and dogs with IE decreased when the data were clustered (Figures 1 and 2), but that difference was still significant for all ROIs (Table 4). Conversely, MTT was not significantly associated with disease status.

Results of $\chi^2$ analyses indicated that both T0 ($\chi^2 [df, 1] = 3,985.0$) and CBF ($\chi^2 [df, 1] = 786.2$; $\chi^2 [df, 10$ with clustering] = 406.08) were significantly ($P < 0.001$ for all comparisons) dependent on ROI selection run. The MTT was significantly ($P < 0.001$ for both comparisons) dependent on ROI selection run ($\chi^2 [df, 1] = 1,549.24$) and AIF ($\chi^2 [df, 1] = 24.704$). None of the perfusion parameters (T0, CBF, and MTT) was dependent on ROI or cerebral hemisphere.

The mean ± SD T0, CBF, and MTT for each of the ROIs for the 12 dogs with IE were summarized (Table 5).
Discussion

In the present study, standard MRI and PWI were used to determine various brain perfusion parameters for dogs with IE, and those parameters were compared with the same parameters determined for healthy dogs of a comparable study. Results indicated that dogs with IE had a greater T0 and lower CBF than healthy dogs, whereas the mean MTT did not differ between dogs with IE and healthy dogs. When the number of blood vessels included in the ROIs during the 5 ROI selection runs were controlled in the CBF analysis (cluster analysis), differences between healthy dogs and dogs with IE were most pronounced in the piriform lobe, thalamus, and temporal cerebral cortex.

For the dogs of the present study, IE was diagnosed subsequent to a history of recurrent generalized tonic-clonic seizures during the 4 weeks prior to examination and by exclusion of other diseases on the basis of unremarkable findings on physical and neurologic examinations, CBC, serum biochemical analysis, CSF analysis, and standard MRI evaluation of the brain. The dogs with IE examined in this study ranged in age from 6 months to 9 years, with a median age of 2 years. In dogs, IE is generally diagnosed between 1 and 5 years of age, although it has been diagnosed in dogs as young as 6 months and as old as 10 years. In older dogs, cryptogenic epilepsy should be considered as a possible differential diagnosis for dogs with epileptic-type seizures without any other abnormalities. Differentiation between idiopathic and cryptogenic epilepsy is difficult. Cryptogenic epilepsy is characterized by seizure episodes that begin as a focal seizure and is considered to be the result of an intracranial lesion. Dogs with a focal onset of seizures as described by the owner or observed on video recordings were excluded from the present study. Also, only dogs for which seizure onset was between 6 months and 3 years were enrolled in the present study; therefore, it is unlikely that any of the study dogs had cryptogenic epilepsy. All dogs were examined interictally. For epileptic patients, the interictal period is defined as the duration between the return to normal consciousness and behavior after seizure activity ceases and the beginning of the next seizure episode. The postictal period is defined as the duration between the end of seizure activity and return to normal conscious-
ness and behavior. Some patients may remain in the postictal phase for 24 hours. All dogs included in this study appeared to have normal mental status on the basis of physical and neurologic examination results and therefore were considered to be in the interictal period when the MRI examination was performed. However, electroencephalographic examinations were not performed, so electrophysiologic alterations in the brain could not be definitively excluded for any of the study dogs.

Cerebral blood flow in the frontal and temporal lobes and subcortical nuclei of dogs > 96 months old is significantly lower than that in younger dogs.33 Two dogs with IE in the present study were > 96 months old; however, exclusion of the data from those 2 dogs from the CBF analyses did not substantially alter our results or conclusions, so we decided to keep them in the analyses.

Anesthetics alter CBF by altering intracranial vasodilation.34–36 The dogs with IE of the present study and the healthy dogs of a similar study25 to which they were compared were anesthetized with the same anesthetic protocol for the MRI examination. Therefore, the effect of anesthetics on the results and conclusions of the present study was believed to be minimal.

For both the healthy dogs25 and dogs with IE, heart rate was indirectly assessed by means of pulse oximetry during the MRI examination. An ECG was not performed because it would have required shaving the hair from the dog’s thorax, a procedure that was denied by most owners of the dogs with IE. Assessment of blood pressure was not possible because it worked only inconsistently in our system. Hemodynamic variables were not assessed statistically and did not appear to subjectively differ between healthy dogs and dogs with IE.

Brain perfusion parameters can also be affected by technical factors such as concentration and dose of contrast medium, pulse sequence, or method of data analysis.37–40 However, we do not believe that technical factors were responsible for the differences observed between the dogs with IE of the present study and healthy dogs of a comparable study25 because the same MRI and data analysis protocols were used for both studies. The correlation coefficients calculated between T0 and TTP, between CBF and T0, and between CBF and MTT for the dogs with IE varied only slightly from those calculated for healthy dogs.

Table 4—Mean ± SD difference in CBF for the healthy dogs and dogs with IE described in Table 1 after the cluster analysis described in Table 2.

<table>
<thead>
<tr>
<th>ROI</th>
<th>Difference in CBF (mL/100 g/min)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate nucleus</td>
<td>28.2 ± 4.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Thalamus</td>
<td>115.1 ± 3.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Piriform lobe</td>
<td>156.8 ± 4.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>9.3 ± 3.8</td>
<td>0.013</td>
</tr>
<tr>
<td>Semioval center</td>
<td>30.2 ± 3.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Temporal cerebral cortex</td>
<td>70.4 ± 3.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>

P values were obtained from least squares fit based on a mathematical model and should be considered cautiously.

Figure 2—Relative CBF (mL/100 g/min) for ROIs in the left (red dots and line) and right (green dots and line) cerebral hemispheres of the healthy dogs (animals 1 through 8 on x-axis) and dogs with IE (animals 9 through 20 on x-axis) of Figure 1. For all ROIs, notice that the CBF tended to decrease from the healthy dogs to the dogs with IE, with the decrease in CBF most pronounced in the caudate nucleus, thalamus, piriform lobe, and temporal cerebral cortex. See Figure 1 for remainder of key.

Table 4—Mean ± SD difference in CBF for the healthy dogs and dogs with IE described in Table 1 after the cluster analysis described in Table 2.

<table>
<thead>
<tr>
<th>ROI</th>
<th>Difference in CBF (mL/100 g/min)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudate nucleus</td>
<td>28.2 ± 4.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Thalamus</td>
<td>115.1 ± 3.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Piriform lobe</td>
<td>156.8 ± 4.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>9.3 ± 3.8</td>
<td>0.013</td>
</tr>
<tr>
<td>Semioval center</td>
<td>30.2 ± 3.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Temporal cerebral cortex</td>
<td>70.4 ± 3.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>

P values were obtained from least squares fit based on a mathematical model and should be considered cautiously.

See Tables 1 and 2 for remainder of key.
CBF (213.1 ± 72.9 mL/100 g/min) for dogs with IE. The hippocampus was also the ROI with the lowest Δ in CBF between healthy dogs and dogs with IE both with and without inclusion of the cluster analysis results. In human patients, the hippocampus has an important role in the pathogenesis of temporal epilepsy, the most common type of epilepsy diagnosed.41 As the hippocampus becomes sclerotic, there is degeneration and selective loss of pyramidal neurons as well as gliosis, sprouting, and reconnection of axons from granulosa cells in the dentate gyrus to other mossy fibers.41,42 In human epileptic patients, the hippocampus in the ictogenic hemisphere is hyperperfused during ictus and hypoperfused during the interictal period relative to the hippocampus in the contralateral hemisphere.9,10 It has been postulated that the increased metabolic activity in the ictogenic hippocampus during ictus leads to anaerobic glycolysis owing to exhaustion of ATP, and hyperperfusion occurs as a compensatory mechanism.20 Subsequent hypoperfusion of the ictogenic hippocampus during the interictal period is believed to be the result of cell loss and sclerosis.23 Results of other studies23,43 involving human patients indicate that the hippocampus has a fairly high CBF during the interictal period that was similar to the CBF observed in the hippocampus for the dogs with IE of the present study. The investigators of one of those studies23 postulated that the high CBF was the result of small hypermetabolic foci within a hypometabolic zone, which could be detected with high-resolution MRI.

A link between hippocampal alterations and epilepsy has been described in veterinary medicine.44-47 In cats with epilepsy, hippocampal changes consistent with acute neuronal degeneration to complete malacia are described and are considered a consequence of ischemia.44,45 Unilateral atrophy of the hippocampus was described in 28 of 58 (48%) dogs with epilepsy.46 However, it remains unclear whether hippocampal changes are the cause or consequence of repeated seizures in veterinary patients with epilepsy.44,45

We can only postulate as to the reasons the hippocampus was the ROI with the highest CBF for dogs with IE and the ROI with the lowest CBF difference between healthy dogs and dogs with IE. The hippocampus is a small structure; therefore, the size of the ROI was excluded as a possible cause for the high CBF. As was assumed for healthy dogs,45 capillaries within the hippocampus might be more tortuous and ramified than those in the other ROIs, which increased the time required for blood to travel through the hippocampus. However, owing to the small size of the hippocampus relative to the other ROIs on dorsal PWIs, we also cannot exclude the possibility that structures not belonging to the hippocampus were included in the ROI and contributed to the observed alterations in CBF.

The semioval center was the ROI with the lowest mean CBF (110.2 ± 39.4 mL/100 g/min) for the dogs with IE of the present study. The semioval center is composed of white matter with a wide-meshed capillary net, which likely contributed to its low blood flow, and was consistent with findings in humans and healthy dogs.48-51 It is also possible that the wide-meshed capillary net of white matter can compensate for changes in CBF better than the close-meshed capillary net of gray matter, and that may also explain why the magnitude of the difference in CBF between healthy dogs and dogs with IE for the semioval center was smaller than that for structures composed primarily of gray matter (eg, temporal cerebral cortex, thalamus, and piriform lobe).

For the dogs with IE of the present study, the mean ± SD CBF was similar for the caudate nucleus (159.3 ± 60.6 mL/100 g/min), piriform lobe (186.7 ± 70.5 mL/100 g/min), and temporal cerebral cortex (173.2 ± 61.8 mL/100 g/min). The fact that those 3 structures are composed primarily of gray matter with a close-meshed capillary net likely explains the similarities in mean CBF and may have contributed to the fairly large Δs in CBF detected between healthy dogs and dogs with IE (caudate nucleus Δ, 28.2 ± 4.3 mL/100 g/min; piriform lobe Δ, 156.8 ± 4.2 mL/100 g/min; and temporal cerebral cortex Δ, 70.4 ± 3.7 mL/100 g/min). However, the large range in the Δs in CBF between healthy dogs and dogs with IE for those 3 structures might also be attributed to the location and size of the respective ROIs. The caudate nucleus is located deep within the brain

### Table 5—Mean ± SD T0, MTT, and CBF within each cerebral hemisphere and overall for 6 ROIs of the brain for the 12 dogs with IE in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hemisphere</th>
<th>Caudate nucleus</th>
<th>Thalamus</th>
<th>Piriform lobe</th>
<th>Hippocampus</th>
<th>Semioval center</th>
<th>Temporal cerebral cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 (s)</td>
<td>Right</td>
<td>31.0 ± 4.6</td>
<td>33.2 ± 4.7</td>
<td>31.3 ± 4.1</td>
<td>32.1 ± 4.3</td>
<td>33.1 ± 4.8</td>
<td>30.6 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>30.5 ± 4.5</td>
<td>33.1 ± 5.5</td>
<td>30.3 ± 4.4</td>
<td>31.6 ± 3.9</td>
<td>33.0 ± 4.5</td>
<td>31.0 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>30.8 ± 4.1</td>
<td>31.1 ± 5.1</td>
<td>30.8 ± 3.6</td>
<td>31.8 ± 4.0</td>
<td>33.0 ± 4.6</td>
<td>30.8 ± 4.9</td>
</tr>
<tr>
<td>MTT (s)</td>
<td>Right</td>
<td>3.5 ± 0.9</td>
<td>3.7 ± 0.9</td>
<td>3.8 ± 0.9</td>
<td>4.2 ± 1.3</td>
<td>3.9 ± 1.1</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>3.4 ± 1.4</td>
<td>3.7 ± 1.1</td>
<td>3.7 ± 1.0</td>
<td>4.0 ± 0.7</td>
<td>3.6 ± 0.8</td>
<td>3.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>3.4 ± 1.2</td>
<td>3.7 ± 1.0</td>
<td>3.7 ± 1.0</td>
<td>4.1 ± 1.1</td>
<td>3.7 ± 1.0</td>
<td>3.6 ± 0.9</td>
</tr>
<tr>
<td>CBF (mL/100 g/min)</td>
<td>Right</td>
<td>152.9 ± 58.7</td>
<td>135.1 ± 42.7</td>
<td>176.5 ± 73.6</td>
<td>200.4 ± 71.3</td>
<td>105.5 ± 41.2</td>
<td>190.7 ± 63.0</td>
</tr>
<tr>
<td></td>
<td>Left</td>
<td>165.8 ± 61.7</td>
<td>139.6 ± 51.8</td>
<td>197.0 ± 65.7</td>
<td>225.7 ± 71.8</td>
<td>114.9 ± 36.8</td>
<td>150.5 ± 55.0</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>159.3 ± 60.6</td>
<td>137.4 ± 46.8</td>
<td>186.7 ± 70.5</td>
<td>213.1 ± 72.9</td>
<td>110.2 ± 39.4</td>
<td>173.2 ± 61.8</td>
</tr>
</tbody>
</table>

See Table 1 for key.

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and does not have any large vessels in close proximity. The size of that ROI was intermediate; therefore, variability among ROI selection runs was fairly small, which may explain why that structure had the lowest $\Delta$ in CBF between healthy dogs and dogs with IE after cluster analysis. In contrast, the piriform lobe is located superficially within the brain and is in close proximity to large vessels on the brain surface that enter the sulci. The piriform lobe ROI was large, and the number of vessels included in that ROI may have varied among the ROI selection runs, which could have affected the calculated CBF. Although the temporal cerebral cortex is also located superficially, the size of its ROI was less than that for the piriform lobe but greater than that for the caudate nucleus, which probably contributed to its mean CBF and $\Delta$ in CBF between healthy dogs and dogs with IE falling between the corresponding values for those other 2 structures. In a study in which brain metabolism as determined by fluorodeoxyglucose-positron emission tomography was compared between healthy and epileptic Logotto Romagnolos, the metabolism for various cortical regions, including the temporal cerebral cortex, was lower in epileptic dogs than in healthy dogs. It seems plausible that hypometabolism may be associated with blood flow alterations. Thus, the decreased CBF in the temporal cerebral cortex of dogs with IE relative to that of healthy dogs may truly reflect a decrease in perfusion. Results of the present study indicated that there was a moderate negative correlation ($\rho$, −0.57; $P < 0.001$) between T0 and CBF.

For the dogs with IE, the mean ± SD CBF of the thalamus (137.4 ± 46.8 mL/100 g/min) was significantly less and the T0 of the thalamus (33.1 ± 5.1 seconds) was significantly greater than the CBF (250.4 ± 51.5 mL/100 g/min) and T0 (24.8 ± 1.9 seconds) of the thalamus for healthy dogs. That finding suggested that dogs with IE have a hypoperfused thalamus relative to the thalamus of healthy dogs. Results of a study in which single-photon emission CT was used to evaluate brain perfusion of dogs with and without epilepsy likewise indicate that the subcortical areas, including the thalamus, of the brains of epileptic dogs are hypoperfused, compared with the subcortical areas of the brains of healthy dogs. Those findings are consistent with the centrencephalic theory, which proposes that structures located deep within the thalamus and brain stem trigger generalized seizures that then project to the cerebral cortex.

In the present study, we decided to report absolute values for the brain perfusion parameters rather than normalizing those values to white matter for 2 reasons. First, it was unknown where perfusion alterations would be detected in dogs with IE. Although white matter alterations were considered unlikely, we were unsure that would prove to be true. Second, one of the aims of this study was to compare brain perfusion parameters between dogs with IE and the healthy dogs of a comparable study, and the brain perfusion parameters of that study were not normalized to white matter. Therefore, we decided to report absolute values and acknowledge that there were drawbacks associated with interfering factors in doing so.

Results of the present study suggested that health status (IE or healthy) had a stronger association with T0 than with CBF. This was surprising because it was expected that the CBF would be significantly altered in diseased brains, whereas the length of the routes to the ROIs (ie, T0) would not be affected. However, the CBF for each ROI was calculated as a function of blood volume scaled to the mass of the ROI. The mass was fairly comparable for each ROI evaluated among healthy dogs but varied substantially among dogs with IE, which likely contributed to less precise measurement of CBF in dogs with IE, compared with healthy dogs. In contrast, T0 was dependent on physiologic parameters and the cross-sectional area of the route to each ROI; it was not dependent on the ROI mass. Nevertheless, the T0 and CBF values reported for this study should be considered cautiously because blood velocity is also dependent on body size. Still, we conjectured that the cross-sectional area of capillaries in the brains of dogs with IE is decreased, compared with that in the brains of healthy dogs, which results in less well-behaved blood circulation in the brain. It should also be noted that we did not determine whether the mean size of the ROI drawn around each anatomic region differed between dogs with IE and healthy dogs.

The size and heterogeneity of the dogs with IE were limitations for the present study. The study population was small; therefore, type II errors could not be excluded. Less stringent inclusion criteria might have allowed for a larger study population. However, the healthy dogs with which the dogs with IE were compared included only 10 Beagles of similar age and body weight, and a large discrepancy in the number of dogs between the 2 studies would have created unacceptable bias. The dogs with IE of the present study were heterogenous in regard to age, breed, and sex. Further research involving large cohorts of dogs of specific breeds that appear to be predisposed to IE (eg, Border Collie) is necessary to identify differences between diseased and healthy dogs.

Another limitation of the present study was the fact that dogs with focal onset of seizures (as determined on the basis of owner consultation or assessment of video recordings) were excluded from the study population. Interobserver agreement in paroxysmal event semiology, especially in recognition of focal seizures, is only fair. Thus, it is possible that some dogs with focal onset of seizures were included in the present study, but the same is true for other studies involving epileptic dogs. Additionally, all ROIs were drawn by 1 investigator (CV); therefore, systematic error was possible. However, that investigator was trained by an experienced investigator (AH) to minimize systematic errors, and if a question arose regarding the margins for a specific ROI, a consensus was achieved between those 2 investigators.

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Therefore, we believe systematic error, particularly in regard to ROI margins, was unlikely.

We believe the results of the present study will provide a basis for future studies. Although we were unable to identify the epileptogenic focus for the dogs with IE of the present study, we did identify significant differences in brain perfusion parameters between dogs with IE and healthy dogs. We also identified drawbacks associated with the use of standardized software for human medicine to determine brain perfusion in dogs. For example, the mass of a specific ROI had a significant effect on some perfusion parameters (eg, CBF), which was unexpected. The size and mass of specific brain structures are fairly similar among adult human patients but vary considerably among dogs of various breeds and sizes. Thus, brain perfusion parameters that are independent of body size need to be identified for more definitive comparisons between dogs of various breeds with and without epilepsy.

Results of the present study indicated that dogs with IE had a significantly lower mean CBF and greater mean T0 than healthy dogs; however, the mean MTT for the 6 brain structures (semeioval center, temporal cerebral cortex, hippocampus, piriform lobe, caudate nucleus, and thalamus) evaluated did not differ significantly between dogs with IE and healthy dogs. Those findings suggested that dogs with IE have decreased blood perfusion of the brain, compared with healthy dogs. However, the brain perfusion parameters for the dogs with IE of this study were reported as absolute values and should not be considered standard values because they were likely affected by various technical factors. Nevertheless, the magnitude of the relationships between the different brain structures examined should be comparable (eg, the CBF for the semeioval center was approximately half the CBF for the hippocampus). Further research is necessary to elucidate blood perfusion alterations and epileptic foci in the brains of dogs with IE to facilitate treatment and surgical management of affected dogs, especially those that have become refractory to antiepileptic drugs.

Acknowledgments

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The authors thank Prof. Martin Kramer (University of Giessen), Prof. Patrick Kircher (University Zürich), and Dr. Matthias Dennler (University Zürich) for technical assistance.

Footnotes

a. Intera Gyroscan, Philips Healthcare, Hamburg, Germany.

b. SENSE-flex M coil, Philips Healthcare, Hamburg, Germany.

c. MR WorkSpace, version 2.6.3.5, Philips Healthcare, Hamburg, Germany.


References


