Description of technique and lower reference limit for magnetic resonance imaging of hippocampal volumetry in dogs

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Objective—To evaluate the use of high-resolution MRI for hippocampal volumetry in dogs and to define a lower reference limit for hippocampal formation (HF) volume.

Animals—20 dogs (with no history of seizures and no underlying structural brain disease) that underwent MRI of the brain.

Procedures—The MRI protocol included a high-resolution T1-weighted 3-D ultrafast gradient-echo sequence aligned in a dorsal plane perpendicular to the long axis of the HF. Images obtained with MRI were retrospectively analyzed by 2 observers (A and B). Intraobserver and interobserver agreement were calculated with the Lin concordance correlation coefficient. Volume measurements of the HF were adjusted for intracranial volume, and a lower 95% reference limit for adjusted HF volume was calculated.

Results—There was substantial intraobserver agreement (Lin concordance correlation coefficient, 0.97 [95% confidence interval {CI}, 0.94 to 0.99]) but poor interobserver agreement (Lin concordance correlation coefficient, 0.63 [95% CI, 0.37 to 0.79]). The lower 95% reference limit for adjusted HF volume was 0.56 cm³ (90% CI, 0.52 to 0.60 cm³) for the right HF and 0.55 cm³ (90% CI, 0.52 to 0.58 cm³) for the left HF.

Conclusions and Clinical Relevance—HF volumes should be adjusted for intracranial volume to account for the large variation in canine skull size. The amount of time required to perform HF volumetry and low interobserver agreement may restrict this technique to research applications, such as the investigation of epileptic patients for hippocampal sclerosis or other cognitive disorders. (Am J Vet Res 2013;74:224–231)

The HF, together with the entorhinal cortex, perihinal cortex, and amygdala, forms part of the mesial temporal lobe. The HF is important for conversion of short-term memory to long-term memory and for learning that requires object recognition.8 Hippocampal atrophy is recognized with a number of human neurologic conditions, such as Alzheimer’s disease,2,3 schizophrenia,4 depression,5 multiple sclerosis,6 and temporal lobe epilepsy.7,8 Hippocampal sclerosis is characterized by hippocampal cell loss and gliosis7,9 and is the most common pathological condition identified in human patients with drug-resistant MTLE.10-12 Antemortem diagnosis of HS is possible with images obtained via tailored MRI protocols that are interpreted by experienced neuroradiologists.13,14 A reduction in hippocampal volume (ie, hippocampal atrophy) is identified in > 90% of patients with HS.15 An affected hippocampus usually has loss of the normal internal architecture and altered signal intensity; it is hyperintense on fluid-attenuation inversion recovery sequences and hypointense on T1-weighted sequences.14 In a randomized, controlled trial of surgery for MTLE, surgical excision of an affected HF resulted in freedom from seizures in 58% of patients, compared with 8% of patients who received medical treatment alone.16 Surgical patients also had improved seizure control and quality of life.16
Of dogs examined at veterinary hospitals because of seizures, approximately half have primary (or idiopathic) epilepsy. Approximately one-fourth of these dogs will be resistant to medical treatment, and many are euthanized because of this lack of response to treatment. A diagnosis of primary epilepsy involves the recognition of recurrent seizures with no identifiable metabolic, inflammatory, or structural brain lesion and no interictal neurologic signs. We hypothesized that some dogs classified as having primary epilepsy may have subtle structural pathological changes that may be detected via improved MRI protocols and interpretation techniques. This would reflect the experience in human medicine after the introduction of high-resolution MRI into clinical neurologic practice.

Numerous case reports indicate the existence of hippocampal pathological changes in epileptic dogs detected during postmortem examination. These changes are seen as acute ischemic damage or chronic change consistent with HS. However, similar to the situation in epileptic humans, not all epileptic dogs will have hippocampal pathological changes. Three studies have been conducted to address the use of MRI for hippocampal volumetry in dogs. These studies have been limited because of a small sample size of a relatively homogenous group of dogs or relatively low-resolution MRI sequences acquired at suboptimal planes. To our knowledge, no reports have provided a description of a robust, repeatable technique for hippocampal volumetry with a high-resolution MRI sequence, nor have investigators attempted to provide a reference interval for hippocampal volume derived from a diverse sample of neurologically normal dogs. Given the wide range of body weights and brain volumes in dogs, a reference interval that accounts for variation in dog size would be desirable. The challenge remains to develop a reliable method of antemortem identification of canine epileptics with HS and to determine whether excision of the affected HF offers improvement in seizure control for this subset of patients.

The purposes of the study reported here were to define an MRI protocol for the optimal assessment of the canine hippocampus, describe the anatomic boundaries of the canine hippocampus (as seen on MRI images), and define the lower reference limit of hippocampal volume from a sample of nonepileptic dogs. The study was intended to test the hypothesis that MRI is a reliable technique for the measurement of hippocampal volume in dogs by determining the intraobserver and interobserver agreement for HF volume determined via MRI.

Materials and Methods

Animals—Hospital records from December 2010 to November 2011 were reviewed to identify MRI brain scans for dogs that did not have epilepsy or evidence of structural brain disease. Two patient groups were established. One group comprised 16 dogs that underwent MRI for conditions such as trigeminal neuritis, vestibular signs, or nasal neoplasia; none of these dogs had evidence of structural brain disease on images obtained via MRI. The second group comprised 4 neurologically normal research dogs that underwent MRI of the brain as part of another, unreported study. The use of research dogs was approved by an institutional animal ethics committee. The groups were combined for analysis.

MRI—All MRI procedures were performed on a 1.5-T MRI scanner. A human knee coil was used for most dogs, and an 8-channel human brain coil was used for dogs with a large head size. A standard brain MRI protocol was used, which included T2-weighted sequences in the sagittal and transverse planes; fluid-attenuation inversion recovery, T2* and diffusion-weighted imaging sequences in the transverse plane; and a T1-weighted 3-D fast SPGR (3-D ultrafast gradient echo) sequence in the dorsal plane.

Optimization of the MRI protocol—Before the present study, a volumetric T1-weighted MRI sequence for assessment of brain anatomy was optimized by our research group. This sequence was acquired in an oblique dorsal plane perpendicular to the long axis of the hippocampus and could be reformatted in any plane with minimal loss of image quality. The plane of acquisition was determined on a patient-by-patient basis, depending on the orientation of the HF Settings for this sequence were as follows: slice thickness, 1 mm; repetition time, 13 milliseconds; echo time, 5.7 milliseconds; inversion time, 600 milliseconds; number of excitations, 2; and flip angle, 15°. Depending on the required number of phase-encoding steps, the scan time for this sequence ranged from 8 to 12 minutes.

Hippocampal volumetry—The HF includes the dentate gyrus, hippocampus, and subiculum. The structure of the HF was determined on the basis of the human literature, and canine anatomic atlases and results of histologic examination of tissue sections and via dissection of anatomic specimens. The HF volume was objectively measured with imaging software on the T1-weighted 3-D fast SPGR sequence for the oblique dorsal plane. Regions of interest around the anatomic borders of the left and right HF were manually traced. To assist delineation of the borders of the HF, concurrent use of the T2-weighted sequence for the transverse plane and the T2-weighted or reformatted T1-weighted sequence for the sagittal plane were used. Calculation of HF volume and generation of a 3-D representation of the HF was performed with imaging software.

Adjustment for ICV—Because of the variation in body weight and brain size, an ANCOVA approach was used to adjust HF volume on the basis of ICV. Intracranial volumes were manually traced from the T1-weighted 3-D fast SPGR sequence reformatted into a sagittal plane with a slice thickness of 1.68 mm. The inner surface of the calvarium was traced. The caudal border included the recesses of the occipital condyles. To demarcate the foramen magnum, a line was traced from the inner margin of the os occipitale to the inner margin of the os basioccipitale. The ventral border included the hypophysis. An ANCOVA approach with the following equation was used:

\[
\text{Volume (adjusted)} = \text{volume (observed)} - (B \times \text{ICV} - \text{ICV}_{\text{mean}})
\]
where $B$ is the slope of the regression line of HF volume regressed on total ICV, ICV is the ICV of a subject, and ICV mean is the overall mean ICV for the study population.

Intraobserver and interobserver agreement—All measurements were made by observers who were unaware of patient identification information and any previous HF volume measurements. Intraobserver agreement was determined for observer A (MEM), who made the measurements by tracing the right HF in all subjects twice, with an interval of at least 2 days between the tracings and measurements. Interobserver agreement was determined by comparison of the first measurement of the right HF made by observer A with measurement of the HF made by observer B (KEC). Observer B underwent a training period under the supervision of observer A. The training included tracing the HF volume of 3 dogs that were not part of the study population. Observer B also was provided with a written description of hippocampal boundaries and a histologic diagram of the cross-section of the HF.

Statistical analysis—Descriptive statistics were calculated for the right and left HF volume, ICV, and adjusted HF volume. Intraobserver and interobserver agreement were evaluated via the Lin CCC. The Lin CCC is the product of a precision component (Pearson correlation coefficient) and a bias correction factor or accuracy component. Lin CCC values were described as almost perfect (> 0.99), substantial (0.95 to 0.99), moderate (0.90 to 0.95), and poor (< 0.90). The 95% limits of agreement were determined by plotting the difference between measurements of observers A and B against the mean of the measurements for both observers. The lower 1-sided 95% reference limit with 90% CI for the right and left adjusted HF volume were calculated via the robust method. Adjusted HF volumes less than the lower 1-sided 95% reference limit were considered indicative of hippocampal atrophy. A statistical program was used for all analyses.

Results

Animals—Twenty dogs were identified that met the inclusion criteria. Body weight had been recorded for 18 dogs (mean body weight, 20.9 kg; range, 1.2 to 38.6 kg). There were 5 Greyhounds, 2 Boxers, 2 medium-sized crossbred dogs, 1 small crossbred dog, and 1 each of Beagle, Bulldog, Cavalier King Charles Spaniel, Chihuahua, Golden Retriever, Griffon Bruxellois, Kelpie, Maltese Terrier, Rottweiler, and Shih Tzu. Two dogs were sexually intact males, 2 were sexually intact females, 3 were neutered males, 11 were neutered females, and 2 dogs were of unknown sex (not recorded).

Anatomic description of the HF—The HF consisted of paired c-shaped structures on the medial aspect of the lateral ventricles (Figure 1). The HF was divided into the head (located rostroventrally) and the body and tail (located caudodorsally). There was variation in orientation of the canine HF, depending on the shape of the brain, with the long axis between 100º and 130º relative to the base of the brain. In brachycephalic dogs, the HF had a more vertical orientation. Internally, the HF was a scrolled structure consisting of the dentate gyrus centrally, the hippocampus (which was divided into subregions...
CA1, CA2, CA3, and CA4), and the subiculum. The internal scrolled structure was not visible in all dogs of the study. The subiculum was continuous with the parahippocampal gyrus. For the present study, the margin of the subiculum and parahippocampal gyrus was defined as a line perpendicular to the tangent of the cortex at the apex of the cortex as it curved caudomedially (Figure 2). The lateral margin of the HF was coated by a thin white matter tract (the alveus). Along the rostromedial margin of the hippocampus, the alveus was continuous with a rostral projection of white matter (the fimbria). At the caudodorsal extent (tail) of the HF, the left and right fimbria combined to form the fornix, which sent white matter projections rostrally into the mamillary bodies. The alveus was included in the HF, but the fimbria and fornix were not. The amygdala was located rostral to the HF and was separated from the HF head by the alveus. The alveus was only visible in high-resolution MRI images with good contrast between the gray matter and white matter and was most evident when sequences were acquired perpendicular to the long-axis of the HF. The tail of the HF curved dorsal to the thalamus. It was sometimes difficult to distinguish the lateral border of the HF from the thalamus; however, the use of point...
region-of-interest crosshairs to colocalize the structure in sagittal and transverse planes assisted with identification of this boundary (Figure 3).

HF volume—There was substantial intraobserver agreement for HF volume, with a Lin CCC of 0.97 (95% CI, 0.94 to 0.99; Figure 4). The Pearson correlation coefficient was 0.976, and the bias correction factor was 0.999. There was poor interobserver agreement for HF volume, with a Lin CCC of 0.63 (95% CI, 0.37 to 0.79; Figure 5). The Pearson correlation coefficient was 0.83, and the bias correction factor was 0.76. Observer B significantly ($P < 0.001$) underestimated HF volume by a mean ± SD of 0.10 ± 0.078 cm$^3$, compared with HF volume estimations for observer A. Evaluation of the limits of agreement between observers A and B revealed a mean difference of 0.10 cm$^3$ (95% limits of agreement, −0.05 to 0.26 cm$^3$; Figure 6).

A positive association was detected between ICV and HF volume (Figure 7). The slope ± SE of the regression line of the right HF volume versus ICV was $0.0043 \pm 0.00065$ cm$^3$, whereas the slope for the regression line of the left HF volume versus ICV was $0.0039 \pm 0.00058$ cm$^3$. A wide range of HF volumes was calculated (Table 1). Mean ICV was 92.73 cm$^3$. Adjustment of HF volume on the basis of ICV via the ANCOVA approach resulted in smaller coefficients of variation, compared with coefficients of variation for nonadjusted HF volumes. Use of the adjusted HF volumes resulted in a calculated lower 95% reference limit for HF volume of 0.56 cm$^3$ (90% CI, 0.52 to 0.60 cm$^3$) for the right HF and 0.55 cm$^3$ (90% CI, 0.52 to 0.58 cm$^3$) for the left HF.

### Discussion

To our knowledge, the study reported here is the first in which investigators have provided a lower 95% reference limit for canine hippocampal volume adjusted for patient size on the basis of ICV. The described volumetry technique and lower reference limit may be used in future investigations of epilepsy and other cognitive disorders in dogs.

Previously, there has been variation in the technique used to identify hippocampal atrophy, which has ranged from comparison with the contralateral hippocampus to comparison with age- and sex-matched control patients. In human patients with medically intractable temporal lobe epilepsy and H5, there is a reduction in unilateral hippocampal volume of up to 33%, compared with the volume for age- and sex-matched control patients. There typically is asymmetry in hippocampal volume, with the right HF being up to 12% larger than the left$^{37}$; in 1 study, investigators

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Table 1—Values for intracranial and hippocampal volumetry derived from MRI images obtained for 20 dogs with no history of seizures and no underlying structural brain disease.

<table>
<thead>
<tr>
<th>Volume</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICV</td>
<td>92.73 ± 24.392</td>
<td>47.65</td>
<td>133.85</td>
<td>28.3</td>
</tr>
<tr>
<td>Right HF</td>
<td>0.67 ± 0.124</td>
<td>0.38</td>
<td>0.85</td>
<td>18.5</td>
</tr>
<tr>
<td>Left HF</td>
<td>0.66 ± 0.113</td>
<td>0.40</td>
<td>0.82</td>
<td>17.1</td>
</tr>
<tr>
<td>Adjusted right HF</td>
<td>0.67 ± 0.087</td>
<td>0.54</td>
<td>0.79</td>
<td>10.0</td>
</tr>
<tr>
<td>Adjusted left HF</td>
<td>0.66 ± 0.060</td>
<td>0.58</td>
<td>0.80</td>
<td>9.1</td>
</tr>
</tbody>
</table>

Values reported are cm$^3$.

*Adjusted on the basis of ICV via an ANCOVA approach.
propose an optimum cutoff value of 13% for the difference between the right and left HF volume. Patients with unilateral MTLE had a 22% reduction in HF volume, compared with HF volume for the contralateral side. Such comparisons are not useful in patients with bilateral hippocampal atrophy.

In dogs, the association between HF volume and body weight limits the usefulness of reference intervals for absolute HF volume in the detection of hippocampal atrophy, and it may be difficult to find sex-, age- and breed-matched control animals for comparison. We attempted to resolve this issue by adjusting HF volume on the basis of the ICV, and the resultant reduction in SD for the adjusted HF volume supported use of this method of volumetry. Normalization of hippocampal volume may be achieved by several methods, including correction on the basis of body weight, cerebral volume, midsagittal cranial area, or ICV. In humans, the ANCOVA approach to correct for ICV is the preferred method and yields the most consistent reduction in SD for the hippocampal volume.

The present study encompassed a wide range of patient sizes, from a Chihuahua that weighed 1.2 kg to a Boxer that weighed 38.6 kg. It also included a wide range of skull shapes, ranging from brachycephalic to dolichocephalic. Other sample populations may yield different reference limits; however, statistical analysis indicated the data for the present study had a relatively narrow 90% CI around the lower 95% reference limit.

The mean HF volume identified in the study (0.67 cm³ for the right HF and 0.66 cm³ for the left HF) is much smaller than that reported in 1 study48 (4.86 cm³) but is closer to that reported in another study29 (0.467 cm³). In the latter study,29 the investigators did not record the body weight of the 9 dogs used in the study; instead, they validated the volume measurement with the volume determined via gross histologic examination. The mean unilateral volume measured in the former study28 is almost twice the mean HF volume measured in humans44 (2.799 cm³ for the right HF and 2.772 cm³ for the left HF), which raises questions about the accuracy of the measurements. The discrepancy between the values reported here and those of that other study28 may be explained by the low spatial resolution and suboptimal alignment of their MRI sequences, which may have led those investigators to include parts of the amygdala and thalamus within their tracings.

The HF has a complex 3-D structure, and it is most visible on oblique dorsal plane images that are aligned perpendicular to the long axis of the hippocampus.37 In humans, images are optimally obtained via an oblique coronal plane perpendicular to the sylvian fissure. The variation in orientation of the canine hippocampus requires that alignment of the oblique dorsal plane of acquisition be determined on a patient-by-patient basis, as was performed in the present study. Despite the variation in canine brain size, slice thickness was maintained constant at an isovolumetric thickness of 1 mm, which represented the limit of resolution for the T1-weighted fast SPGR sequence while achieving an adequate signal-to-noise ratio and scan time.

The use of a constant slice thickness in patients with variable HF sizes may have an impact on the accuracy of HF volume measurement, with greater error expected when measuring smaller volumes. This degree of error may only be quantified by comparison of volume measurements obtained via MRI with volume measurements obtained during postmortem examination, which was not performed in the study population of client-owned dogs. Minimizing the impact of this error requires scanning with the thinnest slice thickness possible; however, maintaining an adequate signal-to-noise ratio is important to allow delineation of HF boundaries. Because of the small size of the structures and difficulty in distinguishing the hippocampal head from the amygdala and the hippocampal tail from the thalamus, volumetric sequences that have a high signal-to-noise ratio and high spatial resolution and with echo time and flip angle optimized for contrasts between gray matter and white matter are required.

A meta-analysis of the medical literature published from 2003 to 2005 on hippocampal volumetry revealed that a wide variety of MRI protocols were used.45 The most popular sequence was the 3-D fast SPGR sequence (35% of reports) and its equivalent, the 3-D MPRAGE (15% of reports).45 Of the 3 reports of hippocampal volumetry in the veterinary literature, proton-density weighted,29 T2-weighted,28 and 3-D MPRAGE30 sequences were used. The study30 that included use of 3-D MPRAGE sequences provided 3-D models of the canine hippocampus that were constructed on the basis of results for 3 healthy Beagles scanned with a 7-T magnet. To the authors’ knowledge, the study reported here represents the first description of the anatomic boundaries and technique for hippocampal volumetry determined with an appropriate sequence at the commonly available magnetic field strength of 1.5 T.

The substantial intraobserver and interobserver repeatability of the technique used in the present study appears promising for the application of manual segmentation of hippocampal volumes in research settings in which a single observer may compare HF volumes between patients or within a single patient over time. Poor interobserver repeatability most likely resulted from inadequate training and an insufficient amount of experience for observer B. Review of manual segmentations made by observer B revealed frequent misinterpretation of the HF boundaries, with inconsistent definition of the boundary between the subiculum and parahippocampal gyrus and a frequent failure to include the entire HF head.

Although a relatively low number of dogs was used, the narrow 90% CI around the lower 95% reference limit indicated that the present study provided a good estimate of the true lower 95% reference limit for HF volume. A larger number of dogs may have allowed better estimation of interobserver agreement; however, most of the difference in agreement was believed to have been attributable to the technique and the insufficient amount of experience of observer B when manually tracing HF volumes, and a larger sample population may not have resulted in improvements.

The absence of gross validation of hippocampal normality and volume may be regarded as a limitation of the present study. However, other authors have reported that with the use of appropriate sequences, MRI volumetry is accurate within 2.5% to 6.8%.8,29 The
study reported here was a retrospective analysis of MRI images of nonepileptic dogs, and it was not possible to confirm normality of the HF via histologic examination.

The volumetry technique used in the present study was a somewhat time-consuming process. It required approximately 50 minutes for an experienced investigator to manually delineate both the HF and ICV. The time required for this technique may be reduced by the use of automated segmentation. However, although automated systems have been used in human studies,46,47 manual tracing remains commonplace. Automatic segmentation techniques have not been established for dogs, in part because of the absence of a canine brain MRI atlas for image coregistration and voxel-based morphometry. Until a more rapid volumetry technique becomes available, quantitative hippocampal volumetry is likely to remain a research tool.

Another limitation of MRI hippocampal volumetry is the difficulty in consistent delineation of the borders of the HF, particularly at the hippocampal head and tail. Careful evaluation of structurally normal hippocampi and colocalization of boundaries in 3 anatomic planes can assist boundary definition, but differences among observers in the definition of HF boundaries26 result in greater interobserver variability than in intra-observer variability. In the human literature, interobserver discrepancies of up to 14% have been reported.48 Although there is no difference in volumes derived from images acquired from humans at 1.5 and 3.0 T,49 the smaller size of the canine HF and differences in contrast between gray matter and white matter in dogs, compared with that in humans, may mean that higher magnetic field strengths will allow more consistent border delineation in canine patients. This may represent an avenue for further investigation.

The study reported here represented an important first step in the investigation of HS in epileptic dogs. Although it is known that there are hippocampal pathological changes in epileptic dogs, we are aware of no systematic studies that have been conducted to determine how widespread the hippocampal pathological changes may be. When evaluating the HF, it is important to acknowledge the requirement for volumetric MRI sequences acquired in an appropriate anatomic plane. We recommend adjusting hippocampal volume measurements on the basis of ICV and use of a lower reference limit of 0.56 cm³ for the right HF and 0.55 cm³ for the left HF to detect potential hippocampal atrophy. Further studies are required to apply both the volumetry technique and subjective hippocampal evaluation to a population of epileptic dogs to identify possible HS and to correlate MRI findings with results of histologic examinations.

References