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PAPER

Linear magnetic resonance imaging measurements of the hippocampal formation differ in young versus old dogs

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Abstract

Age-related hippocampal formation (HF) atrophy has been documented on MRI studies using volumetric analysis and visual rating scales.This retrospective cross-sectional study aimed to compare linear MRI measurements of the HF between young (1–3 years) and old (>10 years) non-brachycephalic dogs, with normal brain anatomy and cerebrospinal fluid (CSF) analysis. Right and left hippocampal formation height (HFH), height of the brain (HB) and mean HFH/HB ratio were measured by two observers on a transverse T2 fluid-attenuated inversion recovery sequence containing rostral colliculi and mesencephalic aqueduct.119 MRI studies were enrolled: 75 young and 44 old dogs. Left and right HFH were greater ($p<0.0001$) in young, while HB was greater in old dogs ($p=0.024$). Mean HFH/HB ratio was 15.66 per cent and 18.30 per cent in old and young dogs ($p<0.0001$). No differences were found comparing measurements between epileptic and non-epileptic dogs.Old dogs have a greater HB; this may represent the different study populations or a statistical phenomenon. Ageing affects HF linear measurements. A reduction of mean HFH/HB ratio between 18.30 per cent and 15.66 per cent should be considered a physiological age-related process of the canine lifespan. The use of mean HFH/HB ratio could be considered for quantifying brain atrophy in elderly dogs.

Introduction

MRI has been used as a non-invasive diagnostic technique for evaluating age-associated changes of the brain in clinically healthy dogs.[1–5] In particular cortical atrophy, dilation of the ventricular system[1–4] and leukoaraiosis[5]—defined as a periventricular white matter T2 hyperintensity—have been described.

The canine hippocampal formation (HF) extends caudally from the pirifom lobe, and, together with its rostral extension, the fornix, forms a C-shaped structure winding round the thalamus. A thin layer of white matter, the alveus, covers its ventricular surface and passes through the fimbria to continue in the fornix. Dorsal to the caudal part of the thalamus, the left and the right HF converge and meet in the midline to form the fornix, which provides a two-way connection between HF and hypothalamus.[6 7] The HF can be divided into three parts: head, body and tail.[8] Furthermore, the following subfields have been also described: dentate gyrus; hippocampus proper, also called the cornu ammonis; and subiculum.[6 7 9]

In humans, HF atrophy has been associated with a number of neurological conditions including Alzheimer's disease,[10–14] Parkinson's disease,[15] Huntington's disease,[16] schizophrenia,[17 18] depression,[19–22] multiple sclerosis[23] and temporal lobe epilepsy (TLE).[24] Hippocampal sclerosis (HS), defined as selective neuronal cell loss with concomitant astrogliosis, is the most common pathological lesion associated with TLE.[25–27] Whether TLE and the associated

Veterinary Record (2019) doi: 10.1136/vr.105243

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Provenance and peer review Not commissioned; externally peer reviewed.

Received October 26, 2018
Revised June 11, 2019
Accepted June 23, 2019

HS represent a discrete form of canine epilepsy remains controversial.[28 29]

In both humans and dogs, volume reduction of the HF has been considered a physiological age-related process, but the magnitude of this change is unclear. In particular, brain MRI has been used to morphologically assess the HF in dogs by manually tracing its boundaries,[3] using volumetric analysis[30–32] and visual rating scales.[33] To date, there are no studies using linear MRI measurements to assess the age-related HF atrophy in dogs.

This study retrospectively compares linear MRI measurements of the HF of young versus old dogs, analysing hippocampal formation height (HFH) and HFH/brain height (HB) ratio to quantify the physiological age-related atrophy.

Materials and methods

This is a retrospective, cross-sectional study. A written owner's consent to use medical records, MRI studies and cerebrospinal fluid (CSF) analysis results for research purposes was obtained at the time of the animal's admission to the hospital.

Clinical records of dogs that underwent brain MRI scan at Dick White Referrals between 2007 and 2014 were retrieved and reviewed initially by a neurology resident (AG) considering the following inclusion criteria: (1) the dog was not brachycephalic; (2) the dog's age was between 1 and 3 years (group Y) or older than 10 years (group O); (3) the MRI study was not performed to investigate behavioural changes; (4) the MRI study did not show any macroscopic evidence of intracranial structural lesions (eg, brain tumour, encephalitis, hydrocephalus and brain asymmetry); (5) a transverse T2 fluid-attenuated inversion recovery (FLAIR) sequence containing rostral colliculi and mesencephalic aqueduct was available; and (6) CSF analysis was performed after MRI to complete the diagnostic work-up by cisterna magna puncture and did not show any abnormalities. A board-certified veterinary neurologist (GBC) and a board-certified veterinary radiologist (OT) reviewed all clinical cases and MRI studies retrieved by the neurology resident to confirm suitability for inclusion.

All MRI studies were performed using a 0.4 Tesla permanent magnet scanner (Aperto Lucente, Hitachi Medical Corporation, Tokyo, Japan) with the dogs' heads positioned in a knee coil. Dogs were positioned in sternal recumbency, and anaesthesia was maintained with isoflurane in oxygen. Sagittal fast spin echo T2 weighted, transverse spin echo T1 weighted, fast spin echo T2 weighted and T2 FLAIR images were acquired (sequences performed and settings are summarised in online appendix 1). T1-weighted images in transverse plane were also repeated immediately after manual intravenous administration of paramagnetic contrast agent (gadobutrol 1 mmol/ml, Gadovist) at a dose of 0.1 ml/kg in all patients. The transverse scans were oriented perpendicular to the sphenoid bone and hard palate.

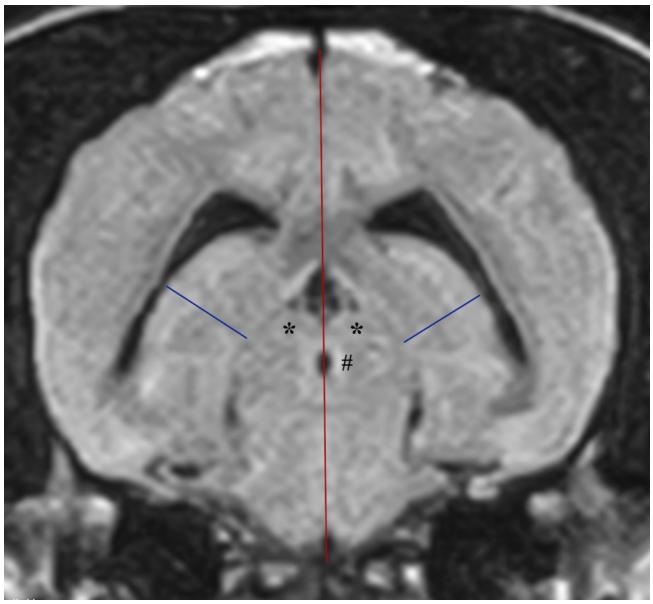

Figure 1 Transverse T2 FLAIR MRI of the brain of a 3-year and 3-month-old jack russell terrier at the level of the mesencephalon, containing both the rostral colliculi (asterisks) and mesencephalic aqueduct (hashtag). Red line=height of the brain. Blue lines=right and left hippocampal formation height.

For the purpose of this study, only one single transverse T2 FLAIR image containing right and left HF bodies, rostral colliculi and mesencephalic aqueduct was selected. For each animal, the HB, and the left and right HFH were measured in mm (figure 1) by two observers (AG and OT) unaware of the dog's signalment. The distance from the inner cortex of the parietal bone to the inner cortex of the sphenoid bone was defined as HB. The distance from the dorsal border of the HF body at the level of its maximum width to the ventral border of the parahippocampal gyrus was defined as HFH. The mean of the right and left HFH (mHFH) was then calculated as: [(right HFH +left HFH)/2]. The ratio between mHFH and HB was calculated as: (mHFH/HB) * 100. All measurements were recorded in mm and saved in an Excel spreadsheet. One year later, AG remeasured the HB, right and left HFH on the same MRI slice in order to assess the intraobserver agreement. Mean HFH/HB ratio was then recalculated.

Only after having obtained all the measurements, breed, gender and body weight were recorded, and dogs were allocated to group Y or O group. Dogs of group Y were further divided into two subgroups: dogs diagnosed with idiopathic epilepsy (epileptic dogs) and non-epileptic dogs. At this stage, AG further reviewed all the MRI images of epileptic dogs in order to identify qualitative MRI features of HS.[34]

Statistical analysis

Considering the results of Noh and others,[35] a total of 28 animals would have been necessary to detect a mean (SD) difference of 1 (1) mm on the mHFH between group Y and O, with a power of 0.8 and an alpha-error of 0.05.

Table 1 Demographic data and measurements of the hippocampal formation in the young (1–3 years old) and the old (>10 years of age) groups

	Young group (n=75)		Old group n=44)		P value
Age (months)	22±9		142±18		<0.0001
Body weight (kg)	20.5±12.5		21±11.2		0.81
Sex	14F, 16FS, 18M, 27MN		0F, 22FS, 3M , 19MN		0.34
	Observer 1	Observer 2	Observer 1	Observer 2	
Left HFH (mm)	8.26±0.81	8.26±0.82	7.33±0.88	7.32±0.89	<0.0001*
Right HFH (mm)	8.25±0.87	8.25±0.87	7.43±0.86	7.43±0.86	<0.0001*
mHFH (mm)	8.26±0.81	8.26±0.81	7.38±0.84	7.38±0.84	<0.0001*
HB (mm)	45.66±3.53	45.65±3.55	47.26±3.96	47.27±3.98	0.024*
mHFH/HB ratio (%)	18.30±1.62	18.10±1.62	15.66±1.66	15.65±1.66	<0.0001*

*P value indicates statistically significant differences between young and old groups and not within each group.
Observer 1=AG, resident in neurology. Observer 2=OT, board-certified veterinary radiologist.
HB, height of the brain; HFH/HB ratio, hippocampal formation to brain ratio; HFH, hippocampal formation height; mHFH, mean hippocampal formation height

Distribution of continuous data was analysed using Pearson and D'Agostino normality test (GraphPad Prism 7.0 c), and Student's t-test or Mann-Whitney U test were applied accordingly. Fisher's exact test was used for analysis of categorical data. Data are reported as mean (SD) or median (range) depending on normality. P<0.05 was considered statistically significant.

Bland-Altman plots were used to evaluate intraobserver and interobserver agreement and are reported as bias and 95 per cent limits of agreement. In particular, intraobserver agreement was assessed comparing HB, right and left HFH measured by the same observer (AG) 1 year apart; interobserver agreement was assessed comparing HB and mHFH measured by two independent observers (AG and OT).

Results

One hundred and nineteen MRI studies that fulfilled the inclusion criteria were assigned to group Y (75 cases) and group O (44 cases). In group Y, 69 dogs were mesaticephalic and 6 dolichocephalic, while in group O, 39 dogs were mesaticephalic and 5 dolichocephalic. Demographic data of dogs included in the two groups are reported in table 1.

Labrador retriever (n=15), jack russell terrier (n=10), english springer spaniel (n=5) and labradoodle (n=4) were the most represented breeds in the group Y. Dogs of this group were diagnosed with idiopathic epilepsy (n=42), idiopathic vestibular syndrome (n=6), idiopathic facial paralysis (n=4), cervical disc disease (n=4), otitis media/interna (n=4, of which two had vestibulochoclear neuritis), suspected intoxication (n=3), masticatory muscle myositis (n=3) and extracranial diseases (n=9, of which five had a retrobulbar disease and four a temporo-mandibular joint disease). Out of 75 dogs of group Y, 42 dogs were included in the epileptic and 33 in the non-epileptic subgroup.

Labrador retriever (n=6), border collie (n=6), border terrier (n=5) and golden retriever (n=5) were the most represented breed in the group O. Dogs of this group were diagnosed with geriatric vestibular syndrome (n=21), otitis media/interna (n=9, of which four had vestibulocochlear neuritis), idiopathic facial paralysis (n=7), transient ischemic attack (n=5) and cervical disc disease (n=2).

While dogs of group O had a statistically greater HB (p=0.024), right and left HFH, mHFH and mHFH/HB ratio were greater (p<0.0001) in group Y (table 1, figure 2). Within the same group, left (p=0.974) and right (p=0.594) HFH were not different. No differences were found comparing measurements between epileptic and non-epileptic dogs of group Y (table 2). No MRI images of epileptic dogs showed qualitative MRI features of HS.

No differences were found between observers; bias and 95 per cent limits of agreement for intraobserver and interobserver agreement are reported in table 3 and graphically showed in figure 3.

Discussion

Both right and left HFH, and mHFH/HB ratio of non-brachycephalic dogs older than 10 years of age are reduced compared with those of dogs of 1–3 years of age.

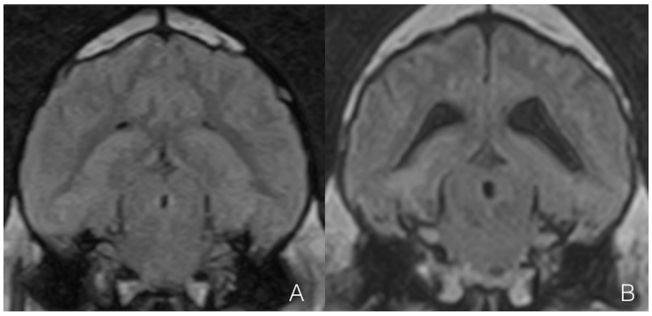

Figure 2 Transverse T2 FLAIR MRI of the brain at the level of the mesencephalon in 2-year and 6-month-old (A) and 12-year (B) labrador retriever dogs. The hippocampal formation size appears greater in the young dog (A) compared with the old one (B). FLAIR, fluid-attenuated inversion recovery.

Table 2 Measurements of the hippocampal formation in the epileptic and non-epileptic young dogs

	Epileptic dogs (n=42)	Non-epileptic dogs (n=33)	P value
Left HFH (mm)	8.30±0.81	8.20±0.84	0.607
Right HFH (mm)	8.31±0.88	8.19±0.86	0.562
mHFH (mm)	8.30±0.81	8.20±0.82	0.569
HB (mm)	46.15±3.14	45.03±3.93	0.1732
mHFH/HB ratio (%)	18.03±1.69	18.25±1.55	0.569

HB, height of the brain; HFH/HB ratio, hippocampal formation to brain ratio; HFH, hippocampal formation height; mHFH, mean hippocampal formation height.
*Significant values.

This confirms that the HF of dogs shrinks with age[30–33] and also supports the use of linear measurements to assess the canine HF dimension on MRI.

Structural and functional changes of the nervous system are common in mammals during ageing. In particular, dogs older than 9 years start to present age-related alterations that gradually progress over the following years. Most often these alterations are clinically silent in uncomplicated ageing.[36] Reported microscopic morphological central nervous system changes in elderly dogs include: diffuse lipofuscin accumulation in the grey matter following an impaired antioxidative defence mechanism;[37] accumulation of amyloid beta plaques in the neuropil and around blood vessels particularly of the cerebral cortex;[36] and microvacuolation and gliosis in the white matter associated with demyelination and secondary axonal loss.[38] Furthermore, neuronal loss associated with apoptosis seems to be more pronounced in specific brain region (ie, prefrontal cortex and medial temporal lobes, particularly HF).[39–41] Moreover, the ability to replace neurons through neurogenesis appears impaired by ageing.[42] Neuronal loss, decreased neurogenesis and white matter myelin loss lead ultimately to age-related brain atrophy.

The atrophic brain is characterised by a reduction in volume and weight, becoming firmer and exhibiting a darker grey-yellow discoloration. The meninges are usually thickened (due to fibrosis or mineralisation) and partially adherent to the skull. The gyri are narrowed and wrinkled with widened sulci and some ventricular dilation.[43] MRI studies performed in clinically healthy dogs confirmed the gross histopathology findings and showed cortical atrophy[1] and ventricular dilation.[1–4] Furthermore, leukoaraiosis in old dogs has been recently reported.[5] Age-related hippocampal atrophy can be caused by neuronal loss and/or impaired neurogenesis.[41 42] In particular, neurogenesis in the HF of Beagle dogs older than 13 years was impaired up to 90 per cent to 95 per cent.[42] Similar results were also reported using double-cortin protein as a marker of newly generated neurons.[44 45]

MRI studies of healthy humans have shown that the hippocampal volume decreases with ageing;[46–49] furthermore, HF volume declines significantly more rapidly than any cortical volume, included the frontal cortex volume.[46] Canine MRI studies analysed the age-related HF appearance by hand-drawing its boundaries,[3] using volumetric analysis[30–32] and visual rating scales.[33] While visual rating scale implies a degree of subjectivity, hippocampal volumetric studies[30–32] might be more objective. However, automatic segmentation technique has not yet been well established in dogs because a canine brain MRI atlas for image coregistration and voxel-based morphometry is lacking. Volumetric technique using manual segmentation is a time-consuming process, and it is mainly employed in a research setting. However, poor interobserver agreement probably related to inadequate training and/or experience of one of the observers has been reported when the volumetric technique with manual segmentation has been used to assess HF in dogs.[32] According to our results, linear MRI measurements could be considered to assess the age-related HF atrophy in dogs as it is easy to perform in a clinical setting even by a non-experienced member of the staff and showed remarkable intraobserver and interobserver agreements. Nevertheless, a direct comparison between linear measurements and volumetric analysis and/or visual rating scales is needed to confirm our findings.

Despite the whole HF atrophying with ageing,[30–33 46–49] it was found that dogs older than 13 years had significant neuronal loss (−30 per cent) in the hilus of the dentate gyrus compared to younger dogs.[41] On MRI, the canine dentate gyrus can be seen at the level of the HF head, using a high field MRI unit and advanced MRI sequence (magnetisation prepared rapid acquisition gradient echo sequence).[50] In our study, linear measurements were obtained at the level of the HF body because HF identification at this level was facilitated by the surrounding CSF. In fact with a low field MRI unit, the boundaries of HF head would have not been distinguishable.

Table 3 Bias and 95 per cent limits of agreement obtained using Bland-Altman plots of right and left hippocampal formation height (HFH) and height of the brain (HB) measured in mm by the same observer (AG, neurology resident) in young and old dogs (intraobserver agreement); HB and mean HFH (mHFH) measured in mm by two different observers (AG and OT, a board-certified veterinary radiologist) in young and old dogs (interobserver agreement)

	Right HFH intraobserver	Left HFH intraobserver	HB intraobserver	HB interindividual	mHFH interindividual
Bias	−0.0008	−0.0066	0.0019	−0.0019	0.0023
95% Lower limit	−0.1016	−0.7503	−0.1494	−03527	−0.0458
95% Upper limit	0.0999	0.737	0.1532	0.3487	0.0505

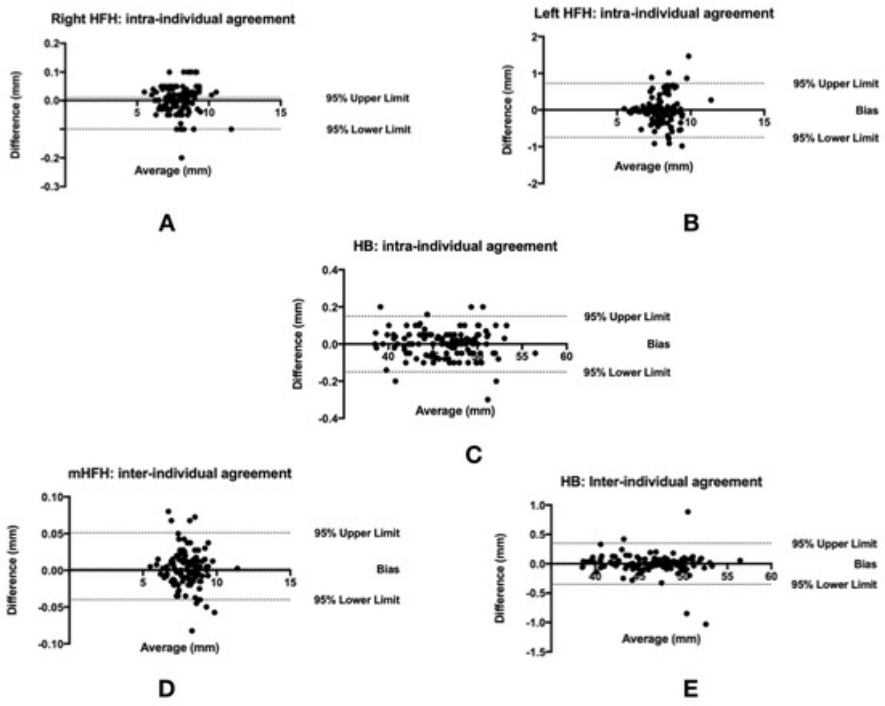

Figure 3 Bland-Altman plots indicating bias and 95 per cent limits of agreement between: (A) right hippocampal formation height (HFH) measured by the same observer (AG, neurologist resident) 1 year apart (intraobserver agreement) in young and old dogs; (B) left HFH measured by the same observer (AG, neurologist resident) 1 year apart (intraobserver agreement) in young and old dogs; (C) height of the brain (HB) measured by the same observer (AG, neurologist resident) 1 year apart (intraobserver agreement) in young and old dogs; (D) mean HFH (mHFH) measured by two different observers (AG and OT, a board-certified veterinary radiologist) (interobserver variability) in young and old dogs; (E) HB measured by two different observers (AG and OT, a board-certified veterinary radiologist) (interobserver variability) in young and old dogs.

Considering the HFH/HB ratio values obtained in the group Y and O, we propose that HFH/HB ratio reduction from 18.30 per cent to 15.66 per cent should be considered physiological across the canine lifespan of non-brachycephalic dogs. In agreement with our results, canine age-related HF shrinkage has been previously documented.[30–33] There is only one single study[3] that did not document HF atrophy with ageing; however, in our opinion, their results might represent a type II statistical error as only 16 dogs were included.

The HB of group O was statistically greater compared with that of group Y, and these results were also confirmed by the measurements taken by OT (a board-certified veterinary radiologist) and the second measurements taken by AG 1 year later. Therefore, it may represent a genuine HB measurement of the different population of dogs included in our study. Another possible explanation is that this represents a statistical phenomenon as a result of the individuals selected for each group. Ideally, a study in which measurements are obtained in the same individuals at young and old age is warranted to better understand if our results are proving a genuine age-related phenomenon or a statistical finding. Being at the denominator, an increased HB in the O group might have amplified the difference of mHFH/HB ratio between the two groups but, in our opinion, not in a clinically significantly matter. Furthermore, despite a greater HB, both right and left HFH were smaller in group O probably because HF declined significantly more rapidly than any cortical structure.[46]

Previous studies have found that cognitively impaired animals had significantly lower interthalamic adhesion size compared with age-matched and size-matched normal dogs.[35 51] To our knowledge, there are no studies rating the HF in cognitive impaired animals; HFH/HB ratio could be a useful tool in a clinical setting to assess the HF in animals affected by canine cognitive dysfunction syndrome (CCDS). In human medicine HF atrophy has been associated with a number of neurological conditions including Alzheimer's disease.[10–14] People affected by Alzheimer's disease showed cognitive decline that has been associated with more pronounced HF atrophy compared to healthy elderly people.[10–12 14] If this is also true in cognitive impaired dogs, in the future, HF measurement may be a valid tool in the diagnosis of CCDS.

In the present study, the HF size of epileptic and non-epileptic dogs was not different. This is in agreement with another study[52] that compared the hippocampal volume of epileptic and non-epileptic animals. Although the HF in epileptic dogs was also measured in a third study,[29] no direct comparison with the non-epileptic control group was done.

TLE is the most common type of epilepsy in people and is characterised by HS.[25 26] In this particular form of epilepsy, the HF represents the epileptogenic zone.[53] Characteristic histopathological features of HS

include dramatic neuron loss, astrogliosis, granule cell dispersion and mossy fibre sprouting that lead to bilateral or monolateral hippocampal atrophy.[54 55] With qualitative MRI, HS can be detected subjectively as volume loss, increase hippocampal signal intensities on T2-weighted and T2 FLAIR images and loss of internal structure.[34] In dogs, it is still controversial whether TLE represents a discrete form of canine epilepsy.[28 29] One study[28] examined the dentate gyrus of healthy dogs and dogs with intractable epilepsy; no differences were seen in the cytoarchitecture between the two groups. These findings suggest that TLE and associated HS are unlikely to be a common cause of medically intractable epilepsy in dogs. In the present study, hippocampal size of epileptic and non-epileptic young animals was similar, and the epileptic dogs did not show MRI features of HS. Absence of HF atrophy and lack of hippocampal hyperintensity may support the hypothesis that TLE is not a common cause of canine epilepsy. However, further studies to support this hypothesis are needed.

As measured in this study, the HFH includes the HF body and the parahippocampal gyrus, which is part of the paralimbic cortex. This can be considered a limitation, but it was performed because using a low field MRI unit it would have not been possible to distinguish the two structures in the majority of the MRI studies. However, as the parahippocampal gyrus (and other paralimbic zones) is not affected by ageing,[56] the height reduction measured in our study should represent a pure HF change.

Brachycephalic breeds were not included in this study because differences in cranial morphology across dog breeds are closely associated with major neuroanatomical changes, especially in brachycephalic dogs.[57] Because of this variability, the cephalic index should have been calculated, but it was not possible due to the lack of essential anatomical landmarks on MRI. Therefore, to minimise the neuroanatomical variations among the population, brachycephalic breeds were excluded. Nevertheless, this should be considered as a limitation.

Anatomical MRI studies are normally conduced on T1-weighted images as it gives clear anatomical details. However, considering the high number of epileptic animals, we used T2 FLAIR images to look for qualitative MRI changes seen with HS. Furthemore, the orientation of the T2 FLAIR transverse images was perpendicular to the sphenoid and hard palate, as the MRI studies were performed before the publication of the veterinary epilepsy-specific MRI protocol.[58] However, the authors believe that the linear measurements can be applicable also on transverse scans planned perpendicular to the long axis of the HF, as recommended by the International Veterinary Epilepsy Task Force.[58] The new angle of transverse images will unavoidably change the measurements of HFH and HB obtained in this study. However, the use of HFH/HB ratio should nullify the difference between the different slice orientations of the transverse images.

In conclusion, right and left HFH and mHFH/HB ratio could be used as linear measurements to assess canine HF dimension and could represents an important first step in the investigation of the HF in elderly dogs. In dogs of 1–3 years of age HFH and mHFH/HB ratio are greater compared with those of dogs over 10 years of age; finally, no difference in linear measurements were found between epileptic and non-epileptic young animals.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/vr.105243).

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