

DOCTORAL (PhD) THESIS

KAPOSVÁR UNIVERSITY

**FACULTY OF AGRICULTURAL AND ENVIRONMENTAL
SCIENCES**

Institute of Diagnostic Imaging and Radiation Oncology

head of the doctoral school

PROF. DR. MELINDA KOVÁCS

correspondent member of the Hungarian Academy of Sciences

supervisor

PROF. DR. IMRE REPA

MAGNETIC RESONANCE IMAGING OF EPILEPTIC DOGS

by

DR. BORBÁLA ARANKA LŐRINCZ

KAPOSVÁR

2017

1. HISTORY AND AIMS OF THE STUDY

Epilepsy is one of the most common neurologic disorders in dogs, affecting about 0.6-7.5% of the canine population. The etiology and clinical appearance of epilepsy are various, therefore the classification, as well as the terminology of the epileptic seizures are considerably complex. Similarly to the human International League Against Epilepsy (ILAE), the International Veterinary Epilepsy Task Force has been established to standardize the definition, terminology and diagnostics of epilepsy in veterinary medicine. According to the recommendations of the latter, the first diagnostic step is the differentiation of epilepsy from other paroxysmal diseases with neurological symptoms (syncope, hypoglycemia, etc.). In the differential diagnosis, the dog characteristics, the clinical history and seizure semiology all play an important role. Regarding their clinical symptoms, there are focal and generalized seizures. Focal seizures are caused by the dysfunction of a smaller brain area, while in case of generalized seizures both hemispheres are involved. Focal seizures could also become generalized. In veterinary medicine – because of the lacking verbal communication with the patient – the diagnosis relies on the owner's description and ideally on video recording, being more challenging than it is in human medicine.

The etiological classification of epilepsy is essential for the proper therapy and accurate prognosis. According to their etiology, there are primary/idiopathic, cryptogenic, structural - secondary/symptomatic, and metabolic - secondary/symptomatic epilepsy. A reactive seizure (i.e. metabolic epilepsy) is a seizure occurring as a natural response of the normal brain to a transient dysfunction of metabolic or toxic nature,

which is reversible when the cause or disturbance is eliminated. Structural - secondary/symptomatic epilepsy is caused by structural brain lesions of vascular, inflammatory, traumatic, hereditary, neoplastic, or degenerative nature. In case of primary/idiopathic and cryptogenic epilepsy, there are no verified structural changes in the brain. By definition, the distinction between these two forms is made by the dog's age at the first seizure – dogs older than 7 years are suspect for cryptogenic epilepsy. The differentiation according to the dog's age only is not reliable; therefore, presenting complementary clinical symptoms (e.g. blindness) is necessary for the diagnosis of cryptogenic epilepsy. The hereditary nature of idiopathic epilepsy is taken for granted or at least very much suspected in certain breeds.

The International Veterinary Epilepsy Task Force created a three-tier system for the diagnosis of primary/idiopathic epilepsy. Here, tier I confidence level is based on a history of two or more unprovoked epileptic seizures occurring at least 24 hours apart, the dog's age at the epileptic seizure onset being between six months and six years, unremarkable inter-ictal physical and neurological examination, and no significant abnormalities in the basic blood and urine tests. Further, tier II confidence level is based on factors listed under tier I, in addition to unremarkable fasting and post-prandial bile acids, MRI of the brain (based on an epilepsy-specific brain MRI protocol), and CSF analysis. Finally, tier III confidence level is based on all factors listed under tier I and tier II, in addition to the identification of characteristic EEG abnormalities for seizure disorders. However, EEG is not a routine diagnostic modality in veterinary medicine yet, its results being not very reliable either.

MRI and CSF-analysis results may be influenced by some transient seizure-related changes. If the latter is suspected, repeated examinations are recommended several weeks later on.

Magnetic resonance imaging plays an important role in the diagnosis of epilepsy. The International Veterinary Epilepsy Task Force recommends a specific epilepsy MRI protocol containing 6 to 7 sequences. Using different MRI techniques, intracranial lesions may be categorized according to their signal intensity, contrast enhancement and localisation – even if not quite matching the accuracy of histopathology.

The hippocampus, located in the medial temporal lobe of the forebrain and belonging to the limbic system, plays an important role in human epilepsy. On one hand, hippocampal sclerosis is the most common pathology underlying pharmacologically intractable cases of the so-called (mesial) temporal lobe epilepsy. In these particular cases, the hippocampus represents the seizure focus – i.e. the epileptogenic lesion - in the brain. On the other hand, there are also further forms of human epilepsy with a dual pathology, where hippocampal abnormalities are associated with extrahippocampal lesions generating the seizure focus. In those cases, hippocampal changes are likely to be secondary to chronic seizuring. Whether temporal lobe epilepsy and the associated hippocampal sclerosis represents a discrete form of canine epilepsy is controversial. According to a study based on histological findings, temporal lobe epilepsy – if present – is not a common cause of medically intractable epilepsy in dogs. However, hippocampal changes, especially hippocampal necrosis is a separate and quite common underlying cause of feline epilepsy, resulting in seizures with orofacial involvement.

Hippocampal changes in epilepsy are manifested as hippocampal atrophy or sclerosis. Histologically, there are evident neuronal loss, gliosis and

secondary shrinking of the hippocampus. With qualitative magnetic resonance imaging, hippocampal sclerosis may be detected subjectively. The MRI criteria of hippocampal sclerosis are volume loss, elevated hippocampal signal intensities on T2-weighted images indicating increased amounts of tissue-free water, and loss of internal structure. There are some other pathologies causing high signal intensities on the water-sensitive MRI sequences such as edema, hyperaemia and inflammation, also detectable in the hippocampus as postictal features. In contrast to hippocampal sclerosis, those changes are reversible, without apparent volume loss.

Due to the subjective nature of the visual (i.e. qualitative) assessment of abnormal T2W hippocampal signals, quantitative MRI methods should be used to reduce subjectivity and increase sensitivity of the detection of hippocampal disease. Visual detection is least reliable in case of mild changes or in bilateral abnormalities. Therefore, quantitative MRI methods such as hippocampal volumetry and T2 relaxometry appear to be more helpful. Tissue hydration is quantified via T2 relaxometry, while hippocampal atrophy is quantified via hippocampal volumetry. In human medicine, hippocampal volumetry and T2 relaxometry are accurate quantitative magnetic resonance tools, that can be used to increase the sensitivity of identifying hippocampal abnormalities above the level of visual assessment, being able to detect subtle changes that otherwise would not be considered to be hippocampal sclerosis.

Using T2 relaxometry, it is also possible to detect seizure-related alterations in the T2 relaxation times of other cerebral structures, such as the temporal lobe white matter or the amygdala. The T2 relaxation times – measured in milliseconds (ms) – are most likely MRI-equipment dependent. Therefore, for a reliable comparison, recording individual and

statistical analysis settings, as well as establishing an equipment-related normal range are recommended.

Aims of the doctoral study:

1. MR imaging of an epileptic dog group to describe their etiologic factors and their frequency.
2. MR imaging of a control dog group without brain related conditions to set a normal range for the volumetry and T2 relaxometry, as well as to compare the control group to the epileptic group with regards to their volumetric and T2 relaxometric results. This control group would also determine the prevalence of brachycephalic skull formation and its possible influences on the epileptic seizures.
3. Volumetric measurements of the brain (hippocampus, lateral ventricle, and hemispherium) in a certain part of the epileptic and of the control groups, as well as the comparison of these two groups.
4. T2 relaxometric measurements of several brain areas (i.e. the hippocampus, and the frontal and temporal white and grey matter) in a certain part of the epileptic and control groups; as well as the comparison of these two groups.
5. Assessing the feasibility and applicability of the volumetric and T2 relaxometric measurements in the diagnosis of epileptic dogs, relying on their results.
6. Developing a new method or parameter based on the volumetric and/or T2 relaxometric results, which could be helpful in diagnosing canine temporal lobe epilepsy.

2. MATERIALS AND METHODS

2.1. Subjects

The MR imaging of 83 epileptic dogs was performed at the Institute of Diagnostic Imaging and Radiation Oncology of the Kaposvár University (Kaposvár, Hungary) between 2010-2014. The clinical diagnosis of epilepsy was made by the referring veterinarians based on the clinical history, dog characteristics, physical and neurological examination and laboratory results. The dogs were presented by referral veterinarians for magnetic resonance imaging to confirm primary or secondary epilepsy. Prior to the MR imaging, laboratory tests were performed in 30 cases, EEG in 6 cases, abdominal ultrasonography in 5 cases, liquor analysis in 3 cases, PET-CT in two cases, radiography and CT scanning in one case. The supplementary examinations were unremarkable, apart from two dogs that showed mildly elevated liver function parameters secondary to long-lasting antiepileptic therapy. Seizure classifications were performed also by the referring veterinarians: there were generalized seizures in 28 and focal seizures in 18 cases, while 11 dogs showed focal seizures with secondary generalization. In another 26 dogs, no seizure classification was performed.

The 31 dogs in the control group have not shown any seizure activity or brain associated neurological signs in their entire life, and were referred to MR imaging for other, not brain related reasons.

2.2. Image acquisition

All dogs underwent general anaesthesia using propofol premedication intravenously (Narcofol®, CP-Pharma GmbH, in 4-7 mg/kgBW bolus injection). Following intubation, the narcosis was maintained using isoflurane-oxygen inhalation (Forene®, AbbVie Deutschland GmbH & Co, 1-5 Vol%, oxygen flow 2-3 l/min).

2.2.1. Qualitative magnetic resonance imaging

The magnetic resonance examinations were performed using a 1.5T MR scanner (Siemens Magnetom Avanto, Siemens, Erlangen, Germany) in ventral recumbency. Identical native MR protocols were used consistently in each case, covering the entire brain: T2W images in transversal (TE/TR=105/2900 ms, SL=3mm), sagittal (TE/TR=105/2900 ms, SL=3mm), and paradorsal planes (TE/TR=105/4520 ms, SL=3mm); fluid-attenuated inversion recovery (FLAIR) images in transversal plane (TE/TR=113/8500 ms, SL=3mm); time of flight (ToF) angiographic images in transversal plane (TE/TR=7.15/25 ms, SL=1mm); T1-weighted thin sliced magnetisation prepared rapid gradient-echo (MP-RAGE) images in sagittal plane (TE/TR=4.24/913 ms, SL=0.9mm), as well as reconstructions in the transversal and paradorsal planes. The field of view (FoV) used for all measurements corresponded to that of 224x320 mm. The paradorsal planes (T2W and T1W MP-RAGE) were oriented perpendicular to the long axis of the hippocampus with planning and placing the paradorsal slice on the sagittal plane – as described and recommended previously. In case of detecting lesions in the native images, post-contrast T1W sequences (0.2 ml/kgBW Dotarem 0,05 mmol/l injection, Guerbet, Villepinte, France) were also performed.

The MR images have been evaluated based on the following criteria: symmetry and possible dilatation of the ventricles, altered size of the hippocampus and amygdala, conformation and/or signal intensity, proportion and localisation of the white and grey matter, presence or absence of focal lesions, signs of inflammation, angiographic changes, or other changes in the post-contrast images. In case of subjectively dilatated ventricles, the brain-ventricle index was calculated as described earlier, to differentiate between asymptomatic ventriculomegaly and symptomatic hydrocephalus. After all these results were taken into consideration, the differential diagnosis of primary or secondary epilepsy was made.

2.2.2. Quantitative magnetic resonance imaging

2.2.2.1. Volumetry

Volumetric measurements were performed in the T1-weighted MP-RAGE images using Amira 6 (FEI Visualization Sciences Group, Mérignac, France). After having loaded the images, transversal and paradorsal reconstructions were made based on the sagittal series. First, the windowing and labeling of the ventricles were performed separately (for the lateral ventricles and for the medial ventricle system containing the 3. and 4. ventricles, as well as the mesencephalic aqueduct) followed by manual alignment of the hippocampi and hemispheria. All recognizable anatomical structures were labeled in all slices. After their demarcation in one of the planes, their boundaries were adjusted in the other two planes (Figure 1).

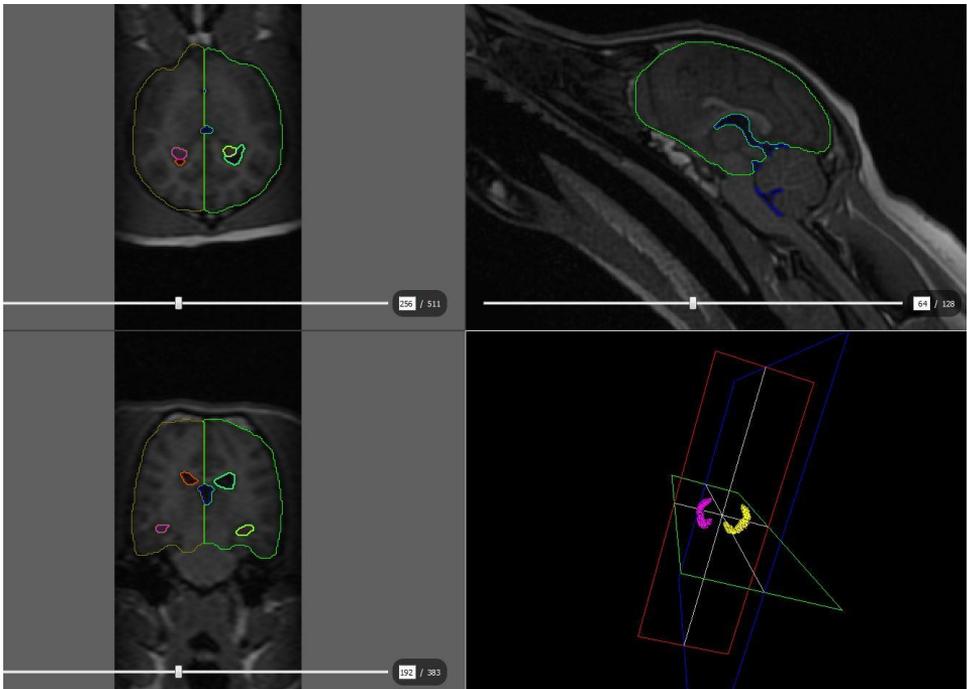


Figure 1.

Manually traced anatomical areas on the dorsal, sagittal and transversal MR-RAGE images and 3D visualisation of the hippocampi (left/right hippocampus – yellow/pink, left/right lateral ventricle – turquoise-blue/red, median ventricle system – blue, left/right hemisphere – green/brown).

2.2.2.2. T2 relaxometry

T2 relaxometry studies were performed during the same anaesthesia and positioning, as well as using the same MR equipment with spin echo sequence (FA: 180 degree; FoV: 230mm×201mm; acquisition matrix: 512×448; SL: 5.0mm; TR: 2450ms; averages: 1). Each slice was scanned with 16 TE values ranging from 22 ms to 352 ms with equal intervals of 22 ms. Eight slices were scanned for each animal with a slice spacing of 10 mm (slice gap: 5mm). T2 maps were calculated from the multi-echo sequence using the Siemens Syngo Dynamic Analysis package. The largest possible regions of interest (ROIs) were traced manually on the T1 PD image series (Figure 2), which has also been derived from the multi-

echo sequence by the software. Boundaries to cerebrospinal fluid (CSF) were avoided to minimize partial volume artefacts. The RoIs were then transferred and measured on the T2 maps (Figure 3) using ITK-SNAP 3.4.0. values of the hippocampus as well as those of the white and grey matter of the temporal and frontal lobes were measured separately in both hemispheres. Figures 2 and 3 show the requirements of using T1 PD images for tracing the RoIs on account of the poor contrast on the T2 maps.

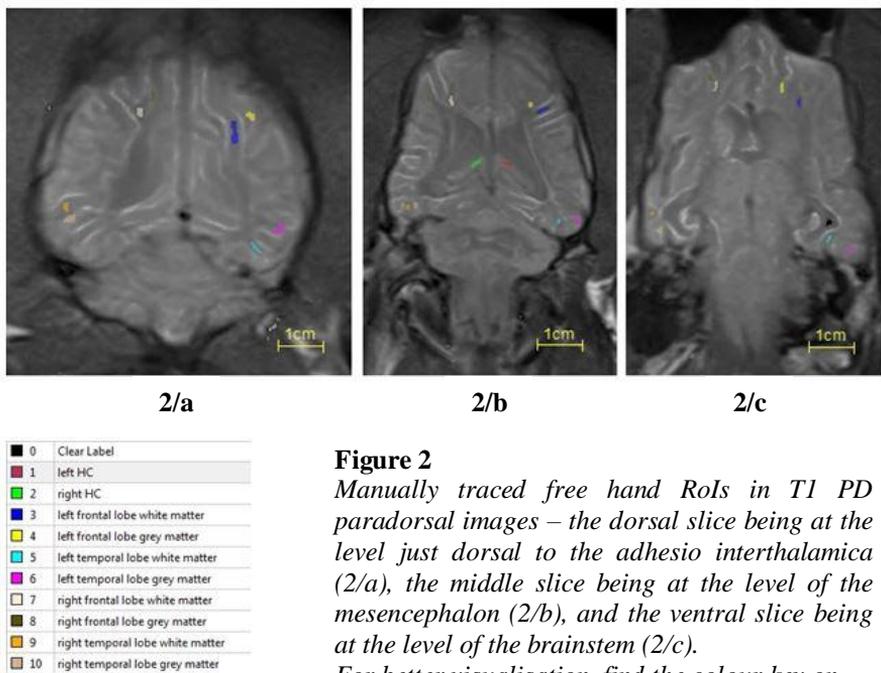
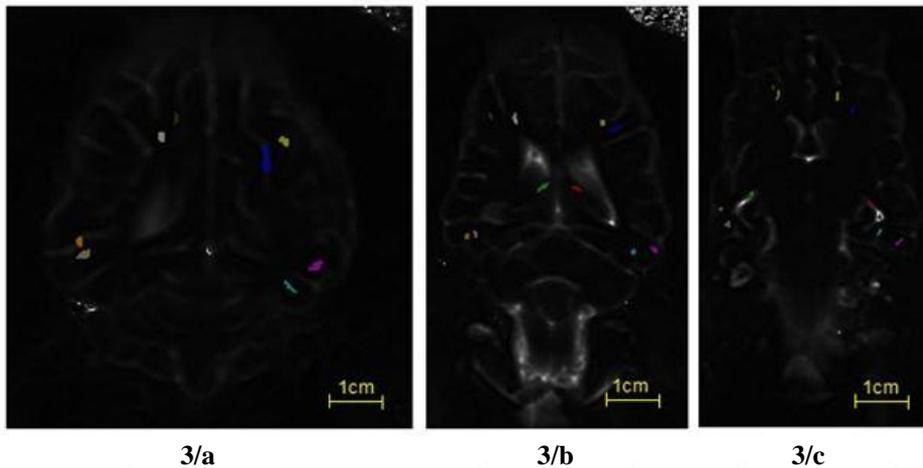


Figure 2

Manually traced free hand RoIs in T1 PD paradorsal images – the dorsal slice being at the level just dorsal to the adhesio interthalamica (2/a), the middle slice being at the level of the mesencephalon (2/b), and the ventral slice being at the level of the brainstem (2/c).

For better visualisation, find the colour key on the left.



0	Clear Label
1	left HC
2	right HC
3	left frontal lobe white matter
4	left frontal lobe grey matter
5	left temporal lobe white matter
6	left temporal lobe grey matter
7	right frontal lobe white matter
8	right frontal lobe grey matter
9	right temporal lobe white matter
10	right temporal lobe grey matter

Figure 3

Transferred RoIs on the T2 maps corresponding to the T1 PD images – the dorsal slice being at the level just dorsal to the adhesio interthalamica (3/a), the middle slice being at the level of the mesencephalon (3/b), and the ventral slice being at the level of the brainstem (3/c).

For better visualisation, find the colour key on the left.

2.3. Statistical analysis

Statistical analysis was performed using the IBM SPSS Version 22 (IBM Corp., Armonk, NY).

2.3.1. Statistical analysis of qualitative MRI

Descriptive statistics have been performed with regards to gender, age, body weight, breed and the presence of ventriculomegaly in the epileptic and control groups, and also within the epileptic group in the primary and secondary subgroups (the allocation of the subjects to the subgroups is based on the MRI results). T-test has been used to describe the distribution of age and body weight, whereas chi-squared test has been

used to look at the distribution of seizure types and at the presence or absence of ventriculomegaly within the epileptic group. Also chi-squared test has been performed to discover the possible influence of gender between the groups.

2.3.2. Statistical analysis of quantitative MRI

2.3.2.1. Volumetry

Median, mean and SD values were calculated for each group and for each anatomical area separately in either hemisphere (lateral ventricles, hippocampi, and hemispheria). In the individual analysis of the hippocampi, after setting the lower and upper reference limits (mean \pm 2 SD) based on control group data, values outside of the range were suspect for hippocampal alterations. The Kolmogorov-Smirnov-test was applied to test the assumption of normal distribution. Apart from the lateral ventricles, our data showed normal distribution, so non-parametric tests were used for the lateral ventricles and parametric tests for the rest of the anatomy. The Pearson's correlation coefficient was used to check correlations in body weight, in age, as well as in brain structure volumes between the groups and also within each group. These correlations involving the lateral ventricles were assessed using the Spearman's correlation coefficient. Hippocampal asymmetric ratio was also calculated. T-test was performed to compare the hippocampal asymmetric ratios between the groups. Asymmetric values with a side difference over 6% were considered as abnormal and their proportions were evaluated separately in the groups.

2.3.2.2. T2 relaxometry

Median, mean and SD values were calculated for each group and for each anatomical area separately in either hemisphere (hippocampus, frontal and temporal white and grey matter). Ratios between hippocampal values and temporal white and grey matter values were explored separately in both groups. A two-step cluster-analysis for groups, genders and skull formation (brachycephalic dogs) was also performed. For individual analysis, the data were z-transformed. Values outside of the range of two SDs were suspect for hippocampal alterations. The Kolmogorov-Smirnov-test was applied to test the assumption of normal distribution. Due to the lack of the latter, these data were analysed using nonparametric tests. The Mann-Whitney-test was used to analyse the differences between groups and between genders, while the Wilcoxon-test was used to compare the values between the left and the right side, as well as among the anatomical regions within each side. The correlation between hippocampal values and age was assessed using Spearman's correlation coefficient.

3. RESULTS

3.1. Results of qualitative MRI

Altogether, 83 dogs – 31 females and 52 males, aged 4.8 ± 3 (0.3 – 14) years, weighing 20.9 ± 14.3 (1.8 – 72) kgs – were included in the epileptic group. The following breeds were represented: Mongrel (n=16), French Bulldog (n=7), Hungarian Vizsla (n=6), Bolognese (n=5), Labrador Retriever (n=4), Golden Retriever (n=3), German Shepherd (n=3), as well as two American Bulldogs, Beagles, Boxers, English Cocker Spaniels, Cavalier King Charles Spaniels, Pugs, Yorkshire Terriers and Dachshunds, respectively; in addition to one American Staffordshire Terrier, Australian Shepherd, Caucasian Shepherd Dog, Coton de Tulear, English Bulldog, German Pointer, Havanese, Howavart, Hungarian Greyhound, Husky, Jack Russel Terrier, Maltese Dog, Miniature Pintscher, Miniature Schnauzer, Moscow Watchdog, Pitbull Terrier, Puli, Pumi, Rhodesian Ridgeback, Rottweiler, Sarplaninac, Spitz and Tervueren, respectively.

Another 31 dogs – 7 females and 24 males, aged 5 ± 2.8 (1-14) years, weighing 18.7 ± 11.5 (3 – 42) kgs – were included in the control group. Here, the following breeds were represented: Mongrel (n=12), Dachshund (n=4), Boxer (n=3), as well as two American Staffordshire Terriers and French Bulldogs, respectively; in addition to one Beagle, Dobermann, Foxterrier, German Shepherd, Miniature Pintscher, Spitz, Welsh Corgi and Yorkshire Terrier, respectively.

In 64 of the 83 epileptic cases, the MRI examinations were unremarkable. In one of latter cases, further laboratory tests were performed after MR imaging. Based on these laboratory results, their final diagnosis was

reactive epileptic seizures secondary to an insulinoma, which is why this particular case was excluded from the study (63/82). In 11 dogs, the ventricle system appeared subjectively to be mildly to moderately dilated. Further, the ventricle-brain index was calculated in all these dogs, which was normal (<0.6) with one single exception: in one American Bulldog with generalized dilatation of the ventricles, the ventricle-brain index was 0.65, which was interpreted as a sign of hydrocephalus. The remaining cases with a normal ventricle-brain index and with no MRI-signs of elevated intraventricular pressure were interpreted as normal variants. Apart from one Labrador Retriever, all dogs showing ventriculomegaly were brachycephalic.

In the subjective visual assessment of the hippocampus, alterations were detected in 10 cases: asymmetry in six cases and mild bilateral shrinkage in four cases. All hippocampi showed normal signal intensity in the T2 sequence.

Finally, the MR examination was unremarkable in 62 dogs (76%), resulting in an imaging diagnosis of primary epilepsy.

In 20 dogs – including the one with the diagnosis of hydrocephalus based on his ventricle-brain index – structural lesions were visible in the MRI. Dogs showing secondary epilepsy were significantly older than those with primary epilepsy ($p=0.001$). No significant differences were found among their body weights. No dogs with secondary epilepsy showed focal seizure symptoms ($p=0.007$).

In the secondary epilepsy subgroup, 10 dogs had the presumptive diagnosis of brain tumour: in nine cases with a cerebral localisation, and in one case within the cerebellum. Cerebellar lesions are not responsible for seizing. In this particular case, obstructive hydrocephalus was caused by the mass effect of the cerebellar lesion (ventricle-brain index:

0.64), secondary leading to seizure activity. In another four cases, a similar mass effect was caused by their forebrain tumours, altering their liquor spaces, that were consequently partially occupied or asymmetrically enlarged. Again in another four cases, an inflammatory background was suspected. In one of the latter, (granulomatous meningoencephalitis (GME) was the most likely diagnosis due to its multifocal appearance. Further imaging diagnosis included Chiari malformation in four cases, as well as porencephaly secondary to a known earlier skull trauma in one single case.

In the control group, ventriculomegaly was present in four dogs, all of those were brachycephalic. Their ventricle-brain index was normal (<0.6). Ventriculomegaly was more frequent in the epileptic group (23%) compared to the control group (13%), although this result was not statistically significant. However, all ventriculomegaly cases with epilepsy were within the secondary epilepsy subgroup ($p=0.037$). In addition to the latter, ventriculomegaly was more often present in the brachycephalic dogs within the control group, as well as in the primary epileptic subgroup within the epileptic group, which trend has not been seen in the secondary epileptic subgroup.

In the subjective visual assessment, asymmetry of the lateral ventricles were also demonstrated. This feature was present in 11% of the primary epileptic subgroup – one of the affected dogs was also brachycephalic –, and in 40% of the secondary epileptic subgroup. Within the control group, the prevalence of lateral ventricle asymmetry was 25%. None of the affected dogs was brachycephalic either in the control group or in the secondary epileptic subgroup.

3.2. Results of quantitative MRI

3.2.1. Volumetry

The numeric results of the volumetric measurements are shown in Table 1, while their visual representation is shown on Figure 4.

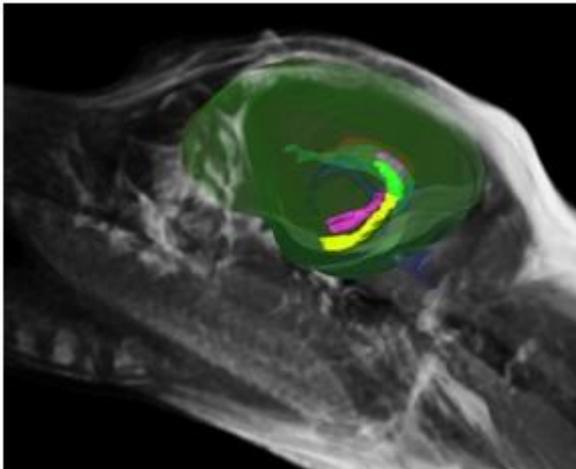
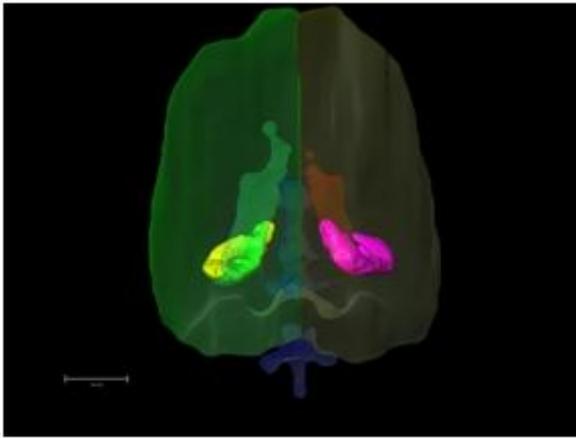
There was a positive correlation between the body weight and the hemispheric volume (both with and without the ventricles, $p < 0.05$), as well as between the hemispheric volume (both with and without the ventricles) and the ipsilateral hippocampal volumes ($p < 0.01$). Further, there was also a significant positive correlation between the hippocampal volumes and the volumes of the lateral ventricles ($p < 0.01$) within the epileptic group. There was no significant correlation among the animal's age and the volumes of any measured brain structures. No statistically significant differences could be found between the hippocampal volumes in the control and in the epileptic group.

There were no statistically significant differences in the hippocampus-hemispherium ratio between the two groups.

Table 1. *Volumetric results*

brain area	volumen (mm ³) mean/median ± SD minimum/maximum	
	control group	epileptic group
left hippocampus	342.73 / 329.87 ± 79.90 193.81 / 495.50	336.74 / 331.87 ± 113.79 115.94 / 573.37
right hippocampus	316.78 / 327.07 ± 85.49 170.61 / 455.34	349.89 / 327.70 ± 142.04 124.10 / 810.79
left lateral ventricle	938.02 / 335.86 ± 1766.96 95.09 / 6915.04	858.97 / 450.99 ± 1137.59 35.26 / 5424.03
right lateral ventricle	721.23 / 224.36 ± 1427.56 17.95 / 5615.83	745.57 / 292.44 ± 1289.95 25.29 / 5330.93
left hemispherium (without ventricle)	38521.10 / 35424.28 ± 11266.91 26087.16 / 66801.16	40493.89 / 41413.98 ± 8901.93 21876.44 / 62527.86
right hemispherium (without ventricle)	36683.00 / 33703.26 ± 9961.84 23724.25 / 62259.62	40444.08 / 42979.71 ± 9779.09 20612.58 / 65828.29
left hemispherium (with ventricle)	39459.12 / 35678.56 ± 12102.60 26647.47 / 66896.25	41352.86 / 42117.44 ± 9419.64 22087.11 / 63939.48
right hemispherium (with ventricle)	37404.24 / 33872.15 ± 10557.44 24193.73 / 62277.57	41222.98 / 43922.62 ± 10308.35 20712.39 / 67182.35

The cut-off value of hippocampal asymmetric ratio was set at 6%, as described earlier. There was no significant difference between the two



groups with regards to the related number of cases either: a hippocampal asymmetric ratio higher than 6% was shown in 60% of the control group and in 77.4% of the epileptic group, both values being above the set cut-off value.

The minimum and maximum hippocampal cut-off values were set based on the control group data (mean \pm 2SD): 182.9 and 502.5 mm³ on the left and 145.8 and 487.8 mm³ on the right side.

Figure 4. 3D-visualisation of the brain structures (left/right hippocampus – yellow/pink, left/right lateral ventricle – turquoise/blue/red, median ventricles – blue, left/right hemispherium – green/brown)

In the epileptic group, there were only seven dogs out of the normal range – two dogs with lower values (one uni- and one bilaterally), and five dogs with higher values (three uni- and two bilaterally). All of these dogs were classified as normal in the subjective visual assessment.

3.2.2. T2 relaxometry

The T2 relaxation times of the different brain areas and groups are shown in Table 2.

Table 2. Results of T2 relaxometry

brain area	T2 relaxation time (ms) (mean/median \pm SD)	
	control group	epileptic group
left hippocampus	127.9 / 117.4 \pm 44.4	144.2 / 113.4 \pm 61.9
right hippocampus	137.5 / 122.9 \pm 38.4	141.6 / 119.0 \pm 55.2
left frontal white matter	120.9 / 113.8 \pm 17.8	115.4 / 109.5 \pm 19.0
right frontal white matter	124.5 / 113.7 \pm 28.7	117.5 / 110.2 \pm 27.8
left frontal grey matter	124.3 / 114.4 \pm 31.5	119.0 / 105.8 \pm 43.4
right frontal grey matter	122.5 / 121.2 \pm 22.8	117.2 / 106.3 \pm 29.4
left temporal white matter	111.8 / 107.0 \pm 19.3	130.2 / 126.7 \pm 33.6
right temporal white matter	115.0 / 105.2 \pm 23.1	142.5 / 119.6 \pm 58.8
left temporal grey matter	110.7 / 104.2 \pm 26.3	107.1 / 99.4 \pm 23.9
right temporal grey matter	106.3 / 97.2 \pm 26.6	112.1 / 101.0 \pm 29.9

Our data showed that the mean hippocampal T2 relaxation times were higher in the epileptic group than those in the control group, but the differences were not statistically significant. Further, no statistically significant differences could be found in the T2 relaxation times of the frontal and temporal lobe white and grey matter between the groups. Although the relaxation times of the temporal lobe white matter did tend to be longer in the epileptic group, these results were not statistically significant either. However, we found that this difference between the

frontal lobe grey matter and the hippocampus was almost statistically significant in the epileptic group ($p=0.063$ on either side), while the same comparison was far from being significant in the control group ($p=0.570$ on the left and $p=0.173$ on the right side). There was no correlation among the hippocampal T2 values and age, gender, or breed in either group. According to the results of the cluster analysis, skull formation – i.e. brachycephalic vs. non-brachycephalic dogs – did not have any effect on the hippocampal T2 relaxation times in either group.

In the individual analysis, values higher than two SDs above the mean of normal controls (216.7 ms on the left and 214.3 ms on the right side) were considered abnormal. Six dogs have been found in the epileptic group showing elevated hippocampal T2 relaxation values: two of them bilaterally (the left/right hippocampal values were 321.8/327.9 ms and 262.6/243.4 ms, respectively), three of them on the left (215.8 ms, 263.6 ms and 258.9 ms) and one of them on the right side (218.4 ms). Two dogs were presented with generalized seizures (one of them showed bilaterally elevated T2 relaxation times), two with focal seizures, one with focal seizures and secondary generalisation (showing bilaterally elevated T2 relaxation times), and one was presented with unknown seizure semiology. One of the observers found two of these six dogs showing abnormal features in their visual assessment. Three of these six dogs showed also elevated T2 relaxation times of the temporal lobe white matter (cut-off values: 161.2 ms on the right and 150.1 ms on the left side), with corresponding lateralisation of the elevated hippocampal values (in one case bilaterally: 161.7 ms on the left and 264.5 ms on the right side, in another case only on the left side: 225.1 ms, and in a third case only on the right side: 301.7 ms).

4. CONCLUSIONS

Visual assessment of qualitative MR images is a subjective and non-accurate method, which may explain its disagreement with the quantitative methods. In human studies, T2 relaxometry has been shown to be a more reliable and more accurate method than volumetry, to detect – essentially mild or bilateral – hippocampal changes. According to our results as previously reported, given the correlation between the body weight as well as the hemispheric and hippocampal volumes, it is controversial to set a lower reference limit for hippocampal volume in dogs. Based on the higher sensitivity of T2 relaxometry for subtle and mild bilateral alterations, dogs showing elevated T2 relaxation times in the individual analysis may belong to a hypothetical subgroup of dogs with temporal lobe epilepsy.

5. NOVEL SCIENTIFIC RESULTS

1. The incidence of ventriculomegaly is not significantly higher in the idiopathic epilepsy subgroup than it is in the control group, while mostly brachycephalic dogs are affected in both groups. Despite the above, dilatated ventricles are more often present in the secondary epileptic group, irrespective of skull formation.

2. The volume of the lateral ventricles does not correlate either with the body weight or with the hemispheric volume, neither in healthy nor in primarily epileptic dogs. The volume of the lateral ventricles may show a wide distribution without any clinical relevance.

3. Hippocampal volume shows a positive correlation with the body weight and also with the hemispheric volume. Setting a lower reference limit for hippocampal volume is not reliable without data correction according to body weight.

4. Methodological definition of T2 relaxometry of the brain, as well as providing with pilot relaxometric results in healthy and epileptic dogs.

5. T2 relaxometry of the brain may be a helpful method to support the diagnosis of canine temporal lobe epilepsy.

6. PROPOSALS

According to our results, temporal lobe epilepsy is supposed to be present in a certain part of the primary epileptic dog group included in this study. T2 relaxometry has been shown to be the most reliable method so far to detect temporal lobe epilepsy, although histological results would be required to provide with a verified, definitive diagnosis in these cases.

The main limitation of this study was the heterogenous subject group. There were several referring veterinarians with different pre-imaging examination protocols, which may have led to miss or to inaccurately interpret some reactive epileptic cases as primary epilepsy. Another limitation is the partially missing seizure classification. Further examinations are needed with a more homogeneous subject group with pre-imaging examinations based on the International Veterinary Epilepsy Task Force Tier I confidence level criteria.

Volumetric measurements may be influenced by the body weight. Therefore, further examinations with body weight correction of the volumetric data are suggested.

The limitations of canine T2 relaxometry are the slice thickness and the interslice gap. Therefore, the T2 relaxometric protocol needs to be optimally adjusted for this purpose.

As volumetric and relaxometric evaluations are quite time consuming methods (0.5-1 hour per subject), this may also represent a limitation for their routine use in the clinical diagnostic practice. Another aim of further examinations or studies may therefore also be to develop an intracranial ratio or certain correlation criteria, which may make it possible to assess abnormal sized hippocampi in the daily routine more quickly, as a significant step forward in diagnosing canine temporal epilepsy.

7. PUBLICATIONS ACCORDING TO THE DOCTORAL THESIS

Papers:

Lőrincz, B.A., Anson, A., Bajzik, G., Csébi, P., Tichy, A., Garamvölgyi, R. (2017): A mágneses rezonanciás képalkotás (MRI) diagnosztikai és differenciáldiagnosztikai szerepe epilepsziás kutyák kivizsgálásában (The role of magnetic resonance imaging /MRI/ in the diagnosis and differential diagnosis of epileptic dogs). *Magyar Állatorvosok Lapja* - accepted

Lőrincz, B.A., Anson, A., Csébi, P., Bajzik, G., Biró, G., Tichy, A., Lőrincz, B.B. and Garamvölgyi, R. (2017): Novel approach to magnetic resonance imaging of epileptic dogs – T2 relaxometry of the brain with emphasised hippocampus. *Acta Veterinaria Hungarica* 65, 185-197.

Lőrincz B.A., Csébi, P., Bajzik, G., Garamvölgyi, R. (2017): Kutyák epilepsziájának kóréletana, etiológiája, tünetei és kivizsgálási lehetőségei - Irodalmi összefoglaló (Pathogenesis, etiology, symptoms and diagnostic methods of canine epilepsy -A review). *Magyar Állatorvosok Lapja*. 139, 413-420.

Reinitz, L.Z., Bajzik, G., Garamvölgyi, R., Benedek, B., Petneházy, Ö., Lassó, A., Abonyi-Tóth, Z., **Lőrincz, B.** and Sótónyi, P. (2017): Linear relationship found by magnetic resonance imaging between cerebrospinal fluid volume and body weight in dogs. *Acta Veterinaria Hungarica*. 65, 1-12.

Reinitz, L.Z., Bajzik, G., Garamvölgyi, R., Petneházy, Ö., Lassó, A., Abonyi-Tóth, Z., **Lőrincz, B.** and Sótonyi, P. (2015): Comparison between magnetic resonance imaging estimates of extracranial cerebrospinal fluid volume and physical measurements in healthy dogs. *Veterinary Radiology and Ultrasound*. 56, 658-665.

Földes, K., **Lőrincz, B.**, Garamvölgyi, R., Bajzik, G., Lelovics, Zs., és Repa, I. (2013): Kutyák cerebellumának mágneses rezonanciás képalkotáson alapuló volumetriai elemzése (The volumetric evaluation of the canine cerebellum by using magnetic resonance imaging). *Magyar Állatorvosok Lapja*. 135, 675-684.

Proceedings:

Lőrincz, B., Anson, A., Csebi, P., Bajzik, G., Biro, G., Tichy, A., Handschuh, S., and Garamvölgyi, R. (2017): Quantitative magnetic resonance imaging of epileptic dogs – hippocampal volumetry and T2 relaxometry. European Veterinary Diagnostic Imaging Congress 2017, Verona, Italy, August 29-September 2, 2017. - accepted

Reinitz, L.Z., Bajzik, G., Garamvölgyi, R., Petneházy, Ö., Lasso, A., Abonyi-Tóth, Z., **Lőrincz, B.**, Benedek, B. and Sótonyi, P. (2014): Volumetric measurements of the canine cerebrospinal fluid using magnetic resonance imaging (MRI). The XXXth Congress of the European Association of Veterinary Anatomists, Cluj-Napoca, Romania, July 23-26, 2014. *Anatomia, Histologia, Embryologia*. 43, 76-77.

Lőrincz, B., Garamvölgyi, R., Bajzik, G., Szántó, A, Nagy, F., Lelovics, Zs., and Repa, I. (2013): MRI-diagnostics of epileptic dogs. „Science of

Sustainability” International Scientific Conference for PhD Students, Győr, March 19-20, 2013. Full paper. p 371-374. (ISBN: 978-963-334-103-2)

Lőrincz, B., Garamvölgyi, R., Nagy, F., Repa, I. (2011): „Epilepsziás” kutyák mágneses rezonancia vizsgálata (Magnetic Resonance Imaging of „epileptic” dogs). Somogyi Egészségügyi Napok (Somogy Health Days), Siófok, September 2-3., p 51-52. (ISBN: 978-963-87916-2-7)

Lőrincz, B., Garamvölgyi, R., Petrási, Zs., Hevesi, Á., Petneházy, Ö., Vajda, Zs. and Repa, I. (2006): MR imaging of syringomyelia: three canine cases. WSAVA 6. Congress, Prague, 11-14 Oct. 2006. p 865. (ISBN: 978-80-902595-4-6)

Abstract:

Lőrincz, B., Garamvölgyi, R., Petneházy, Ö., Benczik, J., Bajzik, G., Lelovics Zs., Repa I.: Chiari-like malformatio előfordulása és MR diagnosztikája kutyákon (MR imaging of Chiari-like malformation in dogs); Magyar Neuroradiológiai Társaság XX. Kongresszusa (20th Congress of the Hungarian Neuroradiological Association), November 8-10., 2012, Eger, Hungary

Oral presentations:

Lőrincz B.: Kutya az MR-ben. Koponya és gerinc vizsgálatok (Canine MRI: skull and spine examinations); Night of Science (Kaposvár University), September 27, 2013. Kaposvár, Hungary

Lőrincz B., Czeibert K.: Tumoros háttérű tüneti epilepsziák MR diagnosztikája (MR diagnosis of brain tumours causing secondary epilepsy). A modern képalkotó eljárások (CT és MR) szerepe az állatorvosi diagnosztikában IV. (Role of modern imaging methods – CT and MRI- in the veterinary diagnosis), February 12, 2012. Kaposvár, Hungary

Lőrincz B., Garamvölgyi R., Petneházy Ö., Benczik J., Bajzik G., Lelovics Zs., Repa I.: Chiari-like malformatio előfordulása és MR diagnosztikája kutyákon (MR imaging of Chiari-like malformation in dogs). Magyar Neuroradiológiai Társaság XX. Kongresszusa (20th Congress of the Hungarian Neuroradiological Association), November 8-10., 2012, Eger, Hungary

Lőrincz B.: MRI az állatorvosi praxisban (MRI in the veterinary praxis). Manninger Days. November 12-13, 2010. Sopron, Hungary

Propagative publications:

Lőrincz B.: Kisállatdiagnosztika a Kaposvári Egyetemen. Modern képalkotó vizsgálatok: MRI és CT (Small animal imaging in the Kaposvár University: CT and MRI). Állati Hírek – Állatkórház Magazin (Animal News), Székesfehérvár, Hungary, 2012. 8, 47-48.