Department of Ethology,
Institute of Biology, Eötvös Loránd University

Department and Clinic of
Surgery and Ophthalmology, University of Veterinary Medicine Budapest

Longitudinal volumetric assessment of ventricle enlargement in dogs trained for functional magnetic resonance imaging (fMRI) studies

Author: Eva Gunde

Supervisors: Dr. Enikő Kubinyi, senior research fellow, Department of Ethology,
Institute of Biology, Eötvös Loránd University
Dr. Kálmán Czeibert, Assistant research fellow, Department of Ethology,
Institute of Biology, Eötvös Loránd University
Dr. Attila Arany-Tóth, Assistant professor, Department and Clinic of Surgery
and Ophthalmology, University of Veterinary Medicine Budapest

Budapest 2018

TABLE OF CONTENTS 2

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS USED

ANOVA	analysis of variance
CSF	cerebrospinal fluid
e.g.	exempli gratia
et al.	et alia
fMRI	functional magnetic resonance imaging
FMRIB	functional magnetic resonance imaging of the brain
FOV	field of view
FSL FMRIB	software library
GM	grey matter
ID	identifier
i.e.	id est
Inc.	incorporated
mm	millimetre
MRI	magnetic resonance imaging
SPSS	statistical package for the social sciences
T	tesla
T_1	longitudinal relaxation time (due to spin-lattice interactions)
TI	inversion time
TR	repetition time
TE	echo time
vs.	versus
WM	white matter

1. *Introduction*

Hydrocephalus is an abnormal accumulation of cerebrospinal fluid (CSF) that requires clinical attention and may result in a diversity of neurological symptoms including visual or auditory impairment, seizures, incoordination, abnormal behavior such as depression, hyper-excitability, and cognitive dysfunction (Vullo, et al., 1997; Ryan, et al., 2014; Laubner, et al., 2015; Thomas, 1999). It is multifactorial disorder with a variety of pathophysiological mechanisms.

CSF is produced by the choroid plexuses in the lateral, third, and fourth ventricles at a constant rate. It is passing through the lateral and the third ventricles, the mesencephalic aqueduct, then from the fourth ventricle, into the subarachnoid space of the brain and the spinal cord via the lateral aperture. The majority of the CSF is absorbed by the arachnoid villi located in the venous sinuses in the subarachnoid space in a passive manner (Thomas, 2010). The production rate is independent from the intracranial pressure, whereas the absorption rate is regulated by pressure differences across the arachnoid villi. They act as valves that halt or increase the absorption in order to keep intracranial pressure between 7-10 cm H_2O in all chambers (Thomas, 2010). Along the main route, CSF is also absorbed via arachnoid surface, capillary walls, and extracranial lymphatic system (Zhao, et al., 2010).

Hydrocephalus can develop when there is a higher pressure gradient between CSF proximal and distal to the obstruction and the alternate pathways are also unable to reduce the CSF (Thomas, 2010; Zhao, et al., 2010), or secondary to thinning parenchyma following trauma, necrosis, or brain tissue atrophy (Thomas, 1999). Several classifications of hydrocephalus exist; 'external' with enlarged subarachnoid space and the 'internal' with ventricle enlargement (Hecht, 2010). It can be a result of increased production of CSF with normal resorption and it may develop due to decreased resorption with normal production rate. The latter form is known to be more common than the formal type, but they both share the feature of increased intracranial pressure (Vullo, et al., 1997; Ryan, et al., 2014; Laubner, et al., 2015; Hecht, et al., 2010; Thomas, 1999). The third type of hydrocephalus is a so-called compensatory type, where the CSF fills the space of neuronal loss and present with normal intracranial pressure (Thomas, 1999; Laubner, et al., 2015). Hydrocephalus can be further categorized as congenital and acquired, the former being prevalent mostly in brachycephalic and small breeds including the Boston terrier, English bulldog, Chihuahua, Pekingese, Yorkshire terrier, and the Maltese at early age (Hecht, et al., 2010; Pilegaard, et al., 2017; De Lahunta, et al., 2014), while the latter form can occur in any breeds and at any age.

Diagnosis of hydrocephalus is based on assessment of the clinical presentation and diagnostic imaging result, such as magnetic resonance imaging (MRI). On the MR image, internal hydrocephalus can present with severe or moderate dilation of ventricles, mostly visible at the lateral ventricles, and often with a well-observable hemispheric asymmetry (Kii, et al., 1997; Esteve-Ratsch, et al., 2001; Woo, et al., 2010; Vullo, et al., 1997).
Despite its characteristic appearance on the MRI however, diagnosis of internal hydrocephalus bares some challenges, as it shares similar imaging features with a clinically silent ventriculomegaly (Vullo, et al., 1997; Ryan, et al., 2014; Laubner, et al., 2015; Thomas, et al., 2010; De Haan, et al., 1994). It is important to highlight that up today, there is still no consensus and no clearly defined delineation between these two conditions. This ambiguity can make arriving to the diagnosis and clinical decisions difficult.
Based on the literature, ventricular enlargement is not always associated with clinical signs of hydrocephalus or increased intracranial pressure. Enlarged ventricles has been demonstrated as a frequent normal morphologic variation in brachycephalic dogs (Ryan, et al., 2014; Vite, et al., 1997; Kii, et al., 1997; Driver, et al., 2013) and in Labradors (De Haan, et al., 1994), as well as it has been associated with normal aging (Head, 2011; Su, et al., 1998; Su, et al., 2005). Further, Thomas (2010) argued that any condition causes thinning of the brain parenchyma, which ultimately leaves a vacant space to be filled by CSF should not be regarded as hydrocephalus (Thomas et al., 2010).

In contrast, a recent study suggested that enlargement of the lateral ventricles seen in brachycephalic dogs might be a consequence of periventricular loss of white matter (WM) tissue due to moderately or intermittently increased intracranial pressure provoking a temporary ischemic effect and ultimately WM loss (Schmidt, et al., 2015). The same research group also noted a reduced periventricular cerebral blood perfusion in clinically sound dogs (Schmidt, et al., 2017), that shown to be decreased in humans with normal pressure hydrocephalus as well (Momjian, et al., 2004; Jeppsson, et al., 2013). They proposed that canine ventriculomegaly is not a physiological variant of ventricular morphology as previously reported, but possibly a preliminary or arrested form of internal hydrocephalus (Schmidt, et al., 2015; Schmidt, et al., 2017).
In ventriculomegaly, accumulation of CSF and distention of the ventricles may occur very slowly that allows the brain to adopt to pathological changes, such as periventricular paren-

chymal thinning and decreased perfusion. Further, to notice the detrimental effects of ventriculomegaly in dogs, a long term accumulation of ischemic insults may be necessary (Laubner, et al., 2015).

It has been demonstrated that hydrocephalus in humans presents with progressive cognitive decline and ultimately dementia (Iddon, et al., 1999). As dogs appear to be ideal model of hydrocephalus (Vullo, et al., 1997; Vullo, et al., 1998; Yamada et al., 1996) and aging related disorders (Katz, 2014; Mazzatenta, et al., 2017; Schutt, et al., 2016; Head, 2013; Tapp, et al., 2005; Tapp, et al., 2004; Su, et al., 2005), it is reasonable to predict that ventriculomegaly present in dogs would result in cognitive or neurological decline over time as well.

To verify these postulations, follow-up studies examining ventricular volume and cognitive performance are needed. To our knowledge, only few follow-up MRI studies assessed ventricular volume changes over time (Hines, et al., 2015; Katz, 2014; Su, et al., 2005). Two of these studies investigated ventricular enlargement only for a short lapse of time and in very young dogs (< 90 weeks). Additionally, the animals underwent drug therapy not relevant to hydrocephalus in both of these studies (Hines, et al., 2015; Katz, 2014). The third study examined healthy dogs between 8-11 years throughout 3 years and found progressive ventricular enlargement with aging (Su, et al., 2005). Cognitive abilities of the participating animals however were not considered.

Assessing cognitive function or preservation of cognitive function in dogs is challenging as sensitive neurocognitive measures are not available for this population as they are for humans. In light of the absence of sensitive canine cognitive indices, perhaps, the way to gain insight of early cognitive decline is to assess and re-assess quantitatively measurable tasks tapping on certain cognitive abilities.

One of the challenges of MR imaging, particularly functional MRI (fMRI), is motion susceptibility. Motion during the scanning time is measured in three directions and it cannot exceed 2 mms in order to acquire adequate quality functional and structural images. Fulfilling this task, i.e. staying completely motionless in the MR machine without any sedation, necessitates variety of cognitive skills, such as sustained attention (e.g. continuous eye contact with the trainer and simultaneously focusing on the stimuli presented) and inhibition (e.g. ignoring dog barks presented as stimuli to the animal). Both attention and inhibition are attributed to frontal lobe activity, and frontal lobe deficits have been shown to be the earliest signs of hydrocephalus in humans (Iddon, et al., 1999). Consequently, we could assume that a measurable performance (i.e. motion during the MR scan time measured by mms) could offer information about the status of the frontal lobe.

Subsequently, we assessed ventricular volume changes in four-year period in fMRI trained dogs. Given the canine lifespan, four years should result in a measurable decline in MRI performance, if ventriculomegaly is associated with the imminent development of clinical signs in silent hydrocephalus.

2. *Objective of the study*

1. We were to investigate whether lateral ventricle enlargement would be measurable among these dogs at the four-year lapse mark.
2. We were to demonstrate that dogs with the ability of staying motionless during the MR scan and being able to repeat the same performance in four years' time, likely do not suffer from cognitive decline regardless of the ventricular enlargement.

The predicted result would mean that ventricular enlargement is not necessarily translate to pathological changes, such as neurocognitive deficits in canine population, which may alter clinical judgment that is based on only neuroimaging results.

3. Methods

3.1 Subjects

Out of the 18 fMRI trained dogs that underwent repeated structural and functional MRI examination, 7 animals, Golden Retrievers (n=3) and Border Collies (n=4), satisfied the criteria of having at least two scans four years apart and being older than 5 years of age at the time of the second scan. The latter criterion was based on previous literature showing increased ventricular dilation after 6 years of age (Su, et al., 1998). The average age at the baseline scanning was 46.3 ± 26 months with a range of 23-60 months and at the second scan 96± 24 months with a range of 76 - 103 months. All animals were family pets and trained by the staff of Department of Ethology of Eötvös Loránd University (Figure 1.).

Figure 1. Participants of the Senior Family Project, fMRI trained dogs.

3.2. Training of the dogs

Description of the training is published elsewhere (Andics, et al., 2016; Andics, et al., 2014), in short, dogs underwent rigorous gradual training using positive reinforcement and social learning at least for six-months. Using a mock scanner, they learnt to climb up a series of steps onto a table, to lay on the table motionless for gradually increasing time, and to get used to the headphones (Figure 2a.). After acquiring these skills, the dogs were trained in the actual scanner, where they habituated to MR noise, get accustomed to the moving table, and to the receiver coil placed on to their heads (Figure 2b.). Besides positive reinforcement in the form of food and praise, social learning was also applied, which involved other dogs observing the training and the feedback of their fellow animal. On average, the animals needed 12 training sessions in the mock scanner and 7 in the MR machine before they could satisfy the criteria of staying motionless (no more than 1mm movement) during the 6 minutes scanning session. During the scanning the dogs were not restrained and could leave the MR suite any time.

The experiment and data collection were approved by the local ethical committee of organization of food safety and animal health agency (Hungarian Directorate for Food Chain Safety and Animal Health XIV-I-001/520-4/2012, Budapest, Hungary).

Figure 2. Training of the dogs.

During training, getting used to the headphones 2a, and in the MR scanner learning to keep eye contact while laying motionless with headphones and the receiver coil secured 2b.

3.3 Imaging technique and analyses

The data were collected using a 3T Philips Achieva magnet (Philips Medical Systems, The Netherlands). As a surface coil, Philips SENSE Flex-M, a two circular element system was used; one was placed under the padding of the scanner table, while the other was secured on the animal's head.

For the structural image Turbo Field Echo sequence was used with the following parameters; repetition time (TR) = 9.9 s, time echo (TE) = 4.6 s; flip angle = 8 °; field of view (FOV) = 255 X 255, slice number = 109 with 1x1x1 mm^3 isovoxel spatial resolution and no gap.

During the scans, the animals kept continuous eye contact with their handler who was in the magnet room as well to assure minimal motion of the animal and to ensure that the animal would not fall asleep. Motion was measured on 3 three planes, transverse (roll), sagittal (pitch), dorsal (yaw). The animals included in the study did not move more than 1 mm in any direction.

Volumetric analyses of the right and left lateral ventricles were completed by software-guided semi-automated tissue type segmentation (Smith, et al., 2004; Jenkinson, et al., 2012) using 0.333 mm off-line isovoxel resolution. FAST (FMRIB's Automated Segmentation Tool) segments MR image of the brain into different tissue types (GM, WM, CSF) based on intensity of a given voxel, and creates probability models that help to determine tissue type around the borders. The program uses bias field-correction for removing intensity variations across space that may be present due to inhomogeneity of the radio-frequency field, applies spatial neighborhood information, creates partial volume model, and based on the information iterates the tissue type segmentation. FAST is robust, reliable, and not sensitive to noise (Zhang, et al., 2001; Figure 3.).

Based on the CSF segmentation templates the lateral ventricles were manually delineated using all 3 planes. The transverse plane was chosen as the primary direction (Figure 4.). The delineated ventricles then were measured counting the number of voxels (3-dimensional pixels).

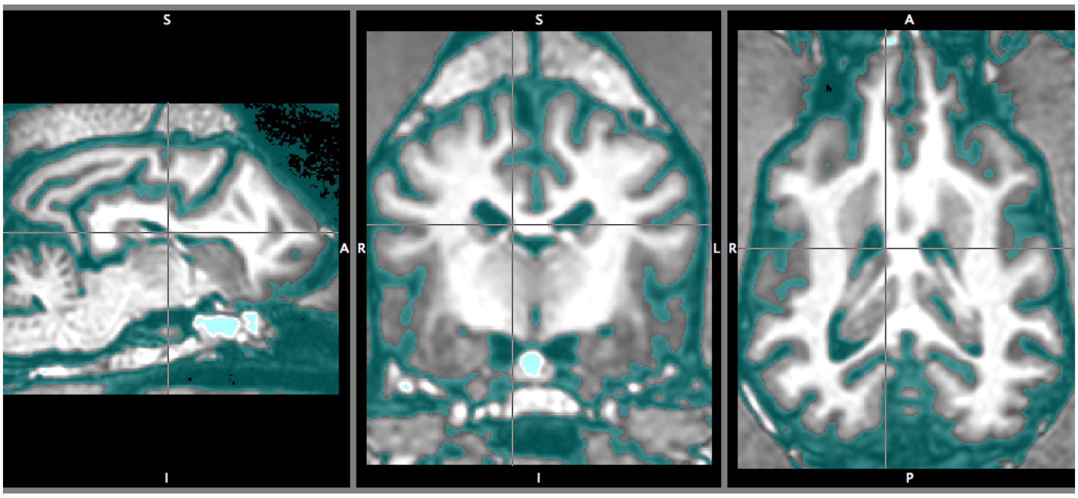

Figure 3. CSF template of the brain.

CSF segmentation on the sagittal, transverse, and on the dorsal plane. On CSF template of the software, the area highlighted in blue signifies the CSF or any other tissue that has the same intensity value. That includes the lateral ventricles.

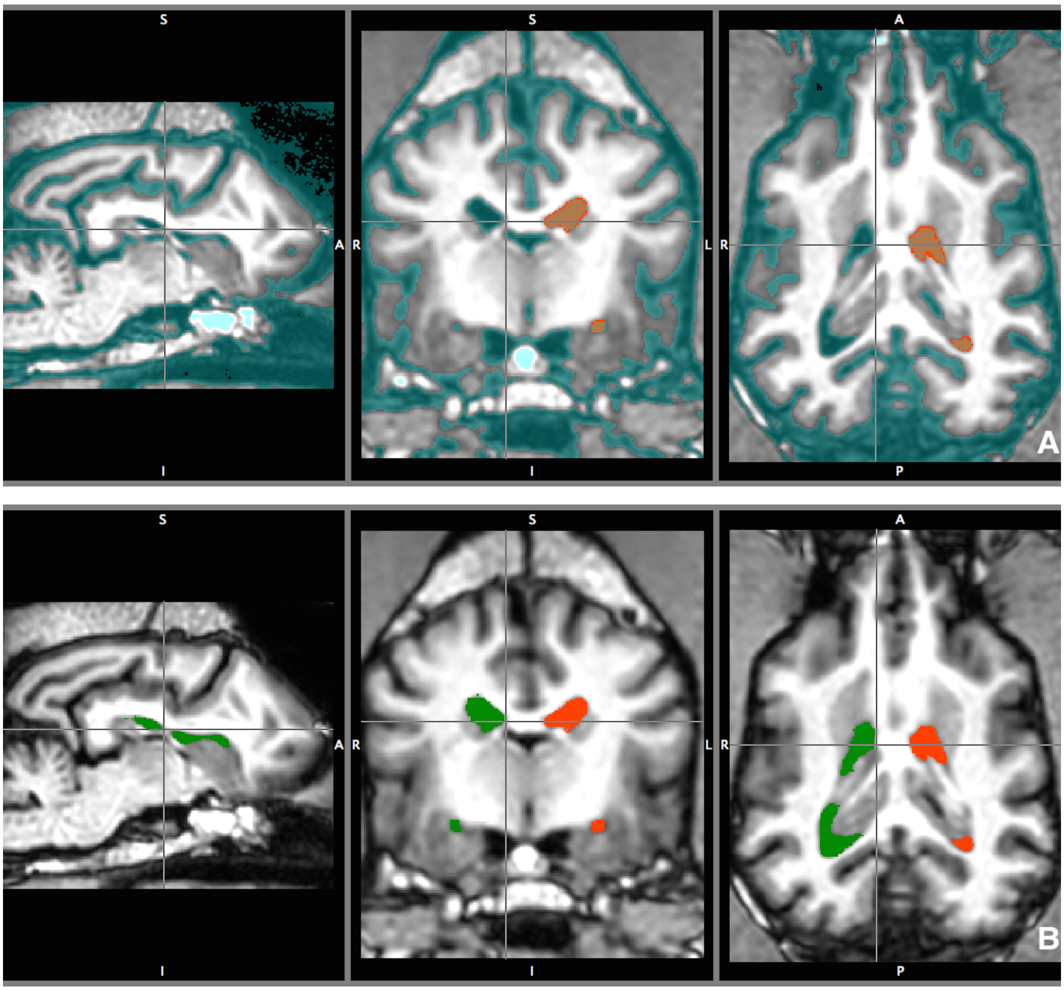

Figure 4. Lateral ventricle template.

Based on the CSF segmentation template, the left ventricle is delineated (red area, 4a), and left and right ventricles delineated (red and green area, 4b) using 3-dimensional tracing. The lateral ventricles are traced separately, creating a template for each of them, the volume of the templates then were calculated.

3.4. Statistical analyses

Statistical analyses were competed using SPSS software. Changes in ventricular volumes over time was calculated using the Friedman test. The Friedman test was chosen because of mandatory criteria of parametric statistics, including normal distribution of the data and homogeneous variance, were not met possibly due to small sample size. Accordingly, the study is better represented by the median vs. the mean. The Friedman test is the non-parametric alternative of one-way ANOVA with repeated measures, in which one group is measured two or more times. This test uses within-subject ranks, which resulted in an eroded data vs. ANOVA would have conveyed.

4. Results

Using the Friedman test, a statistically significant difference between baseline and rescan period was demonstrated $\chi^2 = 7.000$ $p = 0.008$ for left and $\chi^2 = 7.000$ $p = 0.008$ for right side (Figure 5.). One of the dogs had significantly larger baseline and rescan lateral ventricles, when the animal was excluded, χ^2 stayed significant = 6.000 $p = 0.014$ for the left as well as = 6.000 $p = 0.014$ for the right side.

Individual enlargement of the lateral ventricles calculated in percentage revealed however, that the overall enlargement of the seven dogs on the right side (101.1%) was larger vs. on the left side (47.5 %). Results of the individual enlargements are presented in Table 1.

Further, there were considerable individual variations regarding the size of the lateral ventricles (Figure 6.).

Table 1

Ventricular enlargement between 1st and 2nd measurement of the 7 dogs

	Age in months at the time of the scans		**Enlargement in %**	
ID	**1st**	**2nd**	**Left**	**Right**
1	60	115	46.2	46.9
2	39	82	62	24
3	27	78	42.3	94.4
4	99	144	7.9	11.3
5	44	95	71	307.6
6	23	76	41.5	125.5
7	32	80	61.5	98.1
Sum	-		47.5	101.1

Ventricle volumes (left and right) of the seven dogs presented individually and in sum. The enlargement is expressed in percentage comparing volume measured at the 1st and at the 2nd scan.

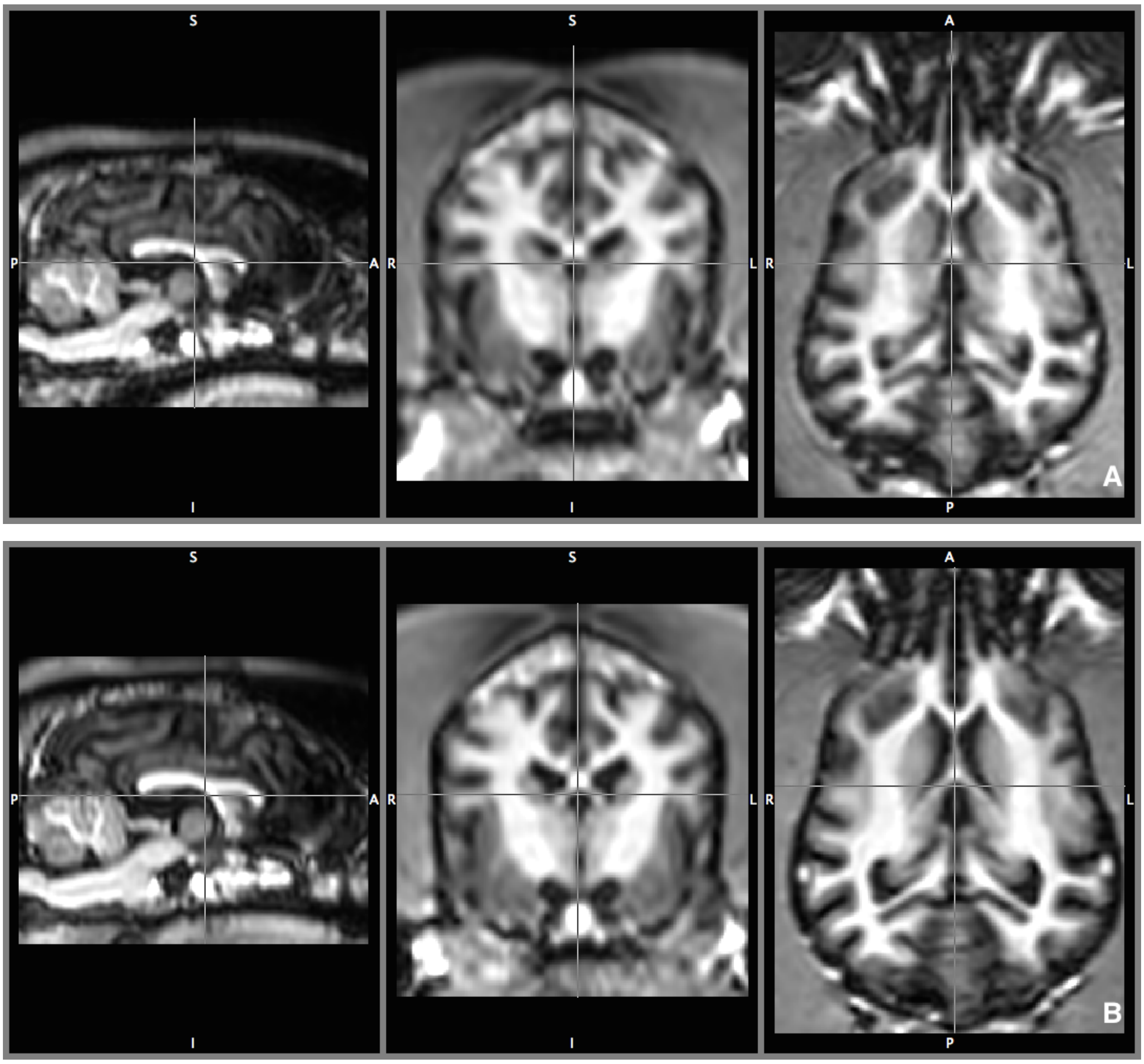

Figure 5. Ventricular changes over time, at the 1st scan and 4 years later at the 2nd scan.

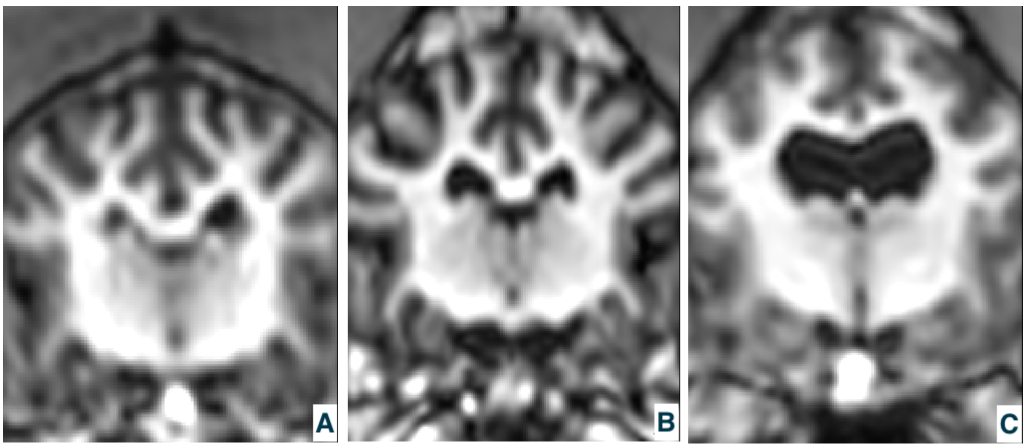

Figure 6. MR images of our three tested dogs.

Mildly (6a), moderately (6b) and extremely enlarged (6c) lateral ventricles are presented.

5. *Discussion*

According to our hypotheses, lateral ventricle enlargement was measurable and significant at the four-year lapse mark in both sides. When expressed in percentage, it was 47.5 % for the left and 101.1 % for the right side for the seven dogs. A previous study noted significant widening of the lateral ventricles over 3-year period (Su, et al., 2005). We chose a 4-year mark to secure not only measurable change in volume, but also that in behavior, if present. The age of the animals, particularly at the rescan (76 months), was also essential, as the genuine volume change should have taken off only after 6 years of age (72 months) (Su, et al., 1998).

The work by Su and colleagues (2005) is the only existing similar follow-up study measuring ventricular enlargement over the years. They reported a lot smaller (4.5%) lateral ventricle enlargement in 8-11 years old dogs compared to our results, however the methods of the two studies are not comparable. The first important difference is that we expressed our enlargement in its actual volume, while in the previous study (Su, et al., 2005), ventricular enlargement was reported as a lateral ventricle / cerebrum ratio. The second important difference is the resolution of the MR images. While the previous study had 1.2-1.5 mm slice thickness, our slice thickness was 0.33 mm, meaning that we had more than 3 times higher resolution, which facilitated superior accuracy.

Although our sample size was small, individual variations in the magnitude of ventricular enlargement is striking. This phenomenon is not related to differences in brain and skull size or its shape, as all dogs included in the study, Golden Retrievers and Border Collies were comparable regarding these characteristics. While ventricular enlargement seems to follow a predictable fashion as a function of age (Su, et al., 1998), our sample suggests that individual variations diverging from the predictable age related widening may not be uncommon. For example, the oldest dog (99 months at baseline -144 months at rescan) had the smallest enlargement, while the youngest (23 months at baseline -76 months at rescan) had close to the largest change. Individual variations in the extent of the widening may be an important consideration in clinical settings, and reminds us the need of development of additional behavioral assessments apart from the currently exercised imaging procedures.

Even though the tested animals cannot be categorized as hydrocephalic dogs, the substantial ventricular enlargement seen in four out of the seven animals may suggest that these animals would present some neurocognitive deficit if ventriculomegaly was truly a preliminary form of hydrocephalus. Neurocognitive implications of hydrocephalus or ventriculomegaly in dogs are still under investigation, but in humans, several cognitive symptoms hallmark to hydrocephalus including attention and inhibition insufficiencies have been confirmed (Iddon, et al., 1999). The animals in our study clearly did not demonstrated any sign of these two deficiencies, according to their MR performance (i.e. monitored motion during scan time). The dogs had to engage constant eye contact with their handler, ensuring that the animal would not fall asleep or move while being scanned. Breaking the eye contact (i.e. attention) would have most likely resulted in at least a minimal (> 1 mm) head motion. Similarly, animals with inhibition deficiencies most likely would have moved in response to the presented stimuli or to the loud scanner. The motion was under 1 mm in every case. Further, there has been no signs or symptoms suggesting central nerves system disorders in any of the participating dogs during the four-year follow-up period. Summing together, it is unlikely that our tested dogs suffered from neurocognitive deficit at the time of the rescan regardless of the substantial ventricular enlargement or ventriculomegaly.

Aside from neuroimaging, the other currently available clinical procedure is neurological testing when ventriculomegaly or hydrocephalus is suspected. Although these clinical assessments are invaluable, they can only measure crude behavioral or neurological impairments, such as gait abnormalities, house soiling, disorientation, sleep-wake cycle disturbances, and interaction changes to list a few (Madari et al., 2015), reflecting significant irreversible brain tissue damage already being present at that point. In order to detect the commencement, the covert manifestation of these disturbances or the lack of them, more sensitive neurocognitive measures tapping on early signs of hydrocephalus or ventriculomegaly are needed.

We attempted to measure certain cognitive skills, such as attention and inhibition via measuring motion in three directions while being scanned. This method is an indirect measure, it is certainly not validated, and does not exhaust assessing all cognitive functions that can be affected in ventriculomegaly, but gives objective information about at least two important cognitive skills. Further, it provides some overview about the capacity of memory of the animals, as the fMRI trained dogs need to perform the very same tasks (staying motionless in the MR scan) many years later with a minimal practice and prompting.

This study is the first of its kind that measures volume of the lateral ventricles in their normal anatomical position in conscious dogs. Although volumetric measurements could be more precise when long sequences are acquired (20-30 minutes or longer) due to gaining better contrast and higher resolution on MR images, these type of imaging however necessitates the anesthesia of the animal. Anesthetics, such propofol and isoflurane, but also the sheer position of the sleeping animal have intracranial pressure modifying properties (Ravussin, et al., 1988; Harrington, et al., 1996; Yamada, et al., 1975), and may influence the volumetric outcome of the ventricles.

The study showed an asymmetry in ventricular enlargement between left (~47 %) and right (~100 %) ventricles. The asymmetry is in accordance with the development of ventriculomegaly or hydrocephalus, but since the sample size is small and the individual variability seems to be large, this difference needs to be further examined. If larger sample shows the same asymmetry, it will be an interesting question to follow why the right hemisphere seems to be more vulnerable to age related changes.

5.1. Summary of the findings

1. The study testifies significant individual variations in ventricle size that may be important consideration in clinics.
2. Our study also points out that even with considerable ventriculomegaly, neurocognitive function can stay intact.
3. This is the first study that measures ventricle volume changes over time in conscious dogs without the potential altering effect of anesthesia.
4. Finally, the need for development of sensitive neurocognitive measures is highlighted.

5.2. Limitation of the study

A major limitation of the study is the small sample size, since the tested animals, (family pets trained for fMRI), represent a special group, therefore it is not clear whether the phenomenon that we are observing is truly representative to the population. Because of the small sample size, parametric statistics was not an option and therefore some information eroded with the available non-parametric statics. These include left-right asymmetry and controlling for age of the animals. The second limitation is the differences in age of the animals at baseline scans. As noted above, aging and the concurrent ventricular enlargement is age dependent. Because of the nature of the statistical analysis, controlling for age was not possible, however, remarkable results regarding individual variations in the magnitude of ventricular enlargement over time is still observable. The third limitation is the scan quality attributed to the surface coil used in these scans. This receiver coil does not have large capacity and therefore contrast is lower on the images, which however did not compromise the volumetric measurement of the lateral ventricles as the region of interest is large and well delineated by its anatomical position.

5.3. Future directions

The most imminent future direction is to improve the MR image quality that allows for volumetric measurement of other brain areas, such the cortical or subcortical regions. Further, determining WM integrity would also provide valuable information about the neurocognitive status of the animals. Matching volumetric results with functional data would give us great opportunity to understand canine brain networks and in turn may help with supplementary tests development to assess behavioral and neurocognitive dysfunction. This study clearly speaks for the importance of such tests and it would be imperative to make these available in clinics without the facility of advanced imaging methods. Research exploring canine brain function may aid to this undertaking.

ABSTRACTS

Recent studies suggest that clinically sound ventriculomegaly in dogs could be a preliminary or an arrested form of the clinically significant hydrocephalus.

There are only a few longitudinal studies measuring ventricular volume changes over time, but assessment of cognitive abilities associated with the change has not been completed. Frontal lobe deficits, such as attention and inhibition have been shown to be the earliest signs of hydrocephalus in humans.

We evaluated changes of ventricular volumes over time in fMRI trained dogs. Staying completely motionless in the MR scanner while awake, entails at least two important cognitive skills; attention and inhibition. Our research question was whether ventricular enlargement developing over time has any effect on canine cognitive ability.

Seven healthy dogs 2-8 years old at the baseline scan and 4 years older at rescan participated in a rigorous and gradual training for staying motionless (< 1 mm) in the MR scanner without any sedation during a 6 minute-long structural MR sequence.

On T1 structural images, volumetric analyses of the lateral ventricles were completed by software guided semi-automated tissue type segmentation.

Ventricular enlargement was significant over time for the left (47.5 %) and for the right side (101.1 %), while the animals' fMRI performance that demanded cognitive indices, such as attention and inhibition, did not change.

Ventricular enlargement arising during normal aging does not necessarily reflect observable pathological changes as dogs with significant ventricular enlargement over the years were still able to execute the fMRI task. This was also the first study which measured volumetrical changes in awake dogs.

Az agykamrák térfogatváltozásának hosszú távú vizsgálata funkcionális mágneses rezonancia vizsgálatra (fMRI) betanított kutyákban

A közelmúlt kutatási eredményei azt mutatják, hogy kutyákban a klinikai tüneteket nem okozó ventriculomegalia bizonyos esetekben súlyos, klinikailag is manifeszt hydrocephalusba progrediálhat, így annak egyfajta előállapotaként értelmezhető. Emberben a hydrocephalus kialakulásának legkorábbi jele a homloklebeny funkciózavara, azaz a figyelem és a gátló funkció károsodása. Kutyákban ezidáig csak néhány longitudinális tanulmány foglalkozott az agykamra térfogatváltozásával és a változáshoz társuló kognitív képességek értékelése sem volt sikeres.

Tanulmányunkban hét, egészséges, 2-8 éves kutyát használtunk, amelyeket arra treníroztak, hogy az MR gépben a 6 perces strukturális szekvencia alatt mozdulatlanul (<1 mm) feküdjenek éber állapotban is. A T1-szerint súlyozott képeken az oldalsó agykamrák térfogatát szoftveresen, az agy szövettípus szerinti szegmentációja segítségével határoztuk meg. A vizsgálatokat 4 évvel később megismételtük. Vizsgáltuk az oldalsó agykamrák térfogatváltozását az idő függvényében. Arra is kíváncsiak voltunk, hogy a kialakult agykamratágulat hatással van-e a kutyák kognitív teljesítményére. Feltételeztük, hogy a figyelem és a gátló funkciók romlása esetén az állatok nem lennének képesek mozdulatlanul feküdni a vizsgálat ideje alatt.

Négy év után az oldalsó agykamrák szignifikáns térfogatnövekedést mutattak a bal oldalon (47.5 %) és a jobb oldalon (101.1 %) is. Valamennyi vizsgálati egyed teljesítette a feladatot, ez alapján feltételezhető, hogy az állatok kognitív teljesítménye nem változott.

Eredményeinkből arra következtetünk, hogy a normális öregedés során fellépő agykamra tágulat nem feltétlenül jár a kognitív funkciók romlásával, mivel a kutyák jelentős agykamratágulat mellett is képesek voltak az fMRI feladat végrehajtására. Ez az első olyan tanulmány, amely az agykamrák longitudinális térfogatváltozását éber kutyákon vizsgálta.

REFERENCES

1. Andics A, Gabor A, Gacsi M, Farago T, Szabo D, Miklosi A. Neural mechanisms for lexical processing in dogs. *Science*. 2016;353(6303):1030-1032. doi: science.aaf3777 [pii].

2. Andics A, Gacsi M, Farago T, Kis A, Miklosi A. Voice-sensitive regions in the dog and human brain are revealed by comparative fMRI. *Curr Biol*. 2014;24(5):574-578. doi: 10.1016/j.cub.2014.01.058 [doi].

3. De Haan CE, Kraft SL, Gavin PR, Wendling LR, Griebenow ML. Normal variation in size of the lateral ventricles of the labrador retriever dog as assessed by magnetic resonance imaging. *Vet Radiol Ultrasound.* 1994;35:83-86.

4. De Lahunta A, Glass EN, Kent M. *Veterinary neuroanatomy and clinical neurology.* 4th ed. St.Louis: Elsevier Health Sciences; 2014.

5. Driver CJ, Chandler K, Walmsley G, Shihab N, Volk HA. The association between chiari-like malformation, ventriculomegaly and seizures in cavalier king charles spaniels. *Vet J*. 2013;195(2):235-237. doi: 10.1016/j.tvjl.2012.05.014 [doi].

6. Esteve-Ratsch B, Kneissl S, Gabler C. Comparative evaluation of the ventricles in the yorkshire terrier and the german shepherd dog using low-field MRI. *Vet Radiol Ultrasound*. 2001;42(5):410-413.].

7. Harrington ML, Bagley RS, Moore MP, Tyler JW. Effect of craniectomy, durotomy, and wound closure on intracranial pressure in healthy cats. *Am J Vet Res*. 1996;57(11):1659-1661.

8. Head E. A canine model of human aging and alzheimer's disease. *Biochim Biophys Acta*. 2013;1832(9):1384-1389. doi: 10.1016/j.bbadis.2013.03.016 [doi].

9. Head E. Neurobiology of the aging dog. *Age (Dordr)*. 2011;33(3):485-496. doi: 10.1007/s11357-010-9183-3 [doi].

10. Hecht S, Adams WH. MRI of brain disease in veterinary patients part 2: Acquired brain disorders. *Vet Clin North Am Small Anim Pract*. 2010;40(1):39-63. doi: 10.1016/j.cvsm.2009.09.006 [doi].

11. Hecht S, Adams WH. MRI of brain disease in veterinary patients part 1: Basic principles and congenital brain disorders. *Vet Clin North Am Small Anim Pract*. 2010;40(1):21-38. doi: 10.1016/j.cvsm.2009.09.005 [doi].

12. Hines CD, Song X, Kuruvilla S, Farris G, Markgraf CG. Magnetic resonance imaging assessment of the ventricular system in the brains of adult and juvenile beagle dogs treated with posaconazole IV solution. *J Pharmacol Toxicol Methods*. 2015;76:55-64. doi: 10.1016/j.vascn.2015.07.011 [doi].

13. Iddon JL, Pickard JD, Cross JJ, Griffiths PD, Czosnyka M, Sahakian BJ. Specific patterns of cognitive impairment in patients with idiopathic normal pressure hydrocephalus and alzheimer's disease: A pilot study. *J Neurol Neurosurg Psychiatry*. 1999;67(6):723-732.

14. Jenkinson M, Beckmann CF, Behrens TE, Woolrich MW, Smith SM. Fsl. *Neuroimage*. 2012;62(2):782-790. doi: 10.1016/j.neuroimage.2011.09.015 [doi].

15. Jeppsson A, Zetterberg H, Blennow K, Wikkelso C. Idiopathic normal-pressure hydrocephalus: Pathophysiology and diagnosis by CSF biomarkers. *Neurology*. 2013;80(15):1385-1392. doi: 10.1212/WNL.0b013e31828c2fda [doi].

16. Katz ML, Coates JR, Sibigtroth CM, et al. Enzyme replacement therapy attenuates disease progression in a canine model of late-infantile neuronal ceroid lipofuscinosis (CLN2 disease). *J Neurosci Res*. 2014;92(11):1591-1598. doi: 10.1002/jnr.23423 [doi].

17. Kii S, Uzuka Y, Taura Y, et al. Magnetic resonance imaging of the lateral ventricles in beagle-type dogs. *Vet Radiol Ultrasound*. 1997;38(6):430-433.

18. Laubner S, Ondreka N, Failing K, Kramer M, Schmidt MJ. Magnetic resonance imaging signs of high intraventricular pressure--comparison of findings in dogs with clinically relevant internal hydrocephalus and asymptomatic dogs with ventriculomegaly. *BMC Vet Res*. 2015;11:181-015-0479-5. doi: 10.1186/s12917-015-0479-5 [doi].

19. Madari A, Farbakova J, Katina S, Smolek T, Novak P, Weissova T, Novak M, Zilka N.. Assessment of severity and progression of canine cognitive dysfunction syndrome using the CAnine DEmentia Scale (CADES). *JABS*. 2015;171:138-145.

20. Mazzatenta A, Carluccio A, Robbe D, Giulio CD, Cellerino A. The companion dog as a unique translational model for aging. *Semin Cell Dev Biol*. 2017;70:141-153. doi: S1084-9521(16)30443-8 [pii].

21. Pilegaard AM, Berendt M, Holst P, Moller A, McEvoy FJ. Effect of skull type on the relative size of cerebral cortex and lateral ventricles in dogs. *Front Vet Sci*. 2017;4:30. doi: 10.3389/fvets.2017.00030 [doi].

22. Ravussin P, Guinard JP, Ralley F, Thorin D. Effect of propofol on cerebrospinal fluid pressure and cerebral perfusion pressure in patients undergoing craniotomy. *Anaesthesia*. 1988;43 Suppl:37-41.

23. Ryan CT, Glass EN, Seiler G, Zwingenberger AL, Mai W. Magnetic resonance imaging findings associated with lateral cerebral ventriculomegaly in english bulldogs. *Vet Radiol Ultrasound*. 2014;55(3):292-299. doi: 10.1111/vru.12123 [doi].

24. Schmidt MJ, Kolecka M, Kirberger R, Hartmann A. Dynamic susceptibility contrast perfusion magnetic resonance imaging demonstrates reduced periventricular cerebral blood flow in dogs with ventriculomegaly. *Front Vet Sci*. 2017;4:137. doi: 10.3389/fvets.2017.00137 [doi].

25. Schmidt MJ, Laubner S, Kolecka M, et al. Comparison of the relationship between cerebral white matter and grey matter in normal dogs and dogs with lateral ventricular enlargement. *PLoS One*. 2015;10(5):e0124174. doi: 10.1371/journal.pone.0124174 [doi].

26. Schutt T, Helboe L, Pedersen LO, Waldemar G, Berendt M, Pedersen JT. Dogs with cognitive dysfunction as a spontaneous model for early alzheimer's disease: A translational study of neuropathological and inflammatory markers. *J Alzheimers Dis*. 2016;52(2):433-449. doi: 10.3233/JAD-151085 [doi].

27. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*. 2004;23 Suppl 1:S208-19. doi: S1053-8119(04)00393-3 [pii].

28. Su MY, Head E, Brooks WM, et al. Magnetic resonance imaging of anatomic and vascular characteristics in a canine model of human aging. *Neurobiol Aging*. 1998;19(5):479-485. doi: S0197-4580(98)00081-5 [pii].

29. Su MY, Tapp PD, Vu L, et al. A longitudinal study of brain morphometrics using serial magnetic resonance imaging analysis in a canine model of aging. *Prog Neuropsychopharmacol Biol Psychiatry*. 2005;29(3):389-397. doi: S0278-5846(05)00006-0 [pii].

30. Tapp PD, Chu Y, Araujo JA, et al. Effects of scopolamine challenge on regional cerebral blood volume. A pharmacological model to validate the use of contrast enhanced magnetic resonance imaging to assess cerebral blood volume in a canine model of aging. *Prog Neuropsychopharmacol Biol Psychiatry*. 2005;29(3):399-406. doi: S0278-5846(05)00007-2 [pii].

31. Tapp PD, Siwak CT, Gao FQ, et al. Frontal lobe volume, function, and beta-amyloid pathology in a canine model of aging. *J Neurosci*. 2004;24(38):8205-8213. doi: 10.1523/JNEUROSCI.1339-04.2004 [doi].

32. Thomas WB. Hydrocephalus in dogs and cats. *Vet Clin North Am Small Anim Pract*. 2010;40(1):143-159. doi: 10.1016/j.cvsm.2009.09.008 [doi].

33. Thomas WB. Nonneoplastic disorders of the brain. *Clin Tech Small Anim Pract*. 1999;14(3):125-147. doi: S1096-2867(99)80030-9 [pii].

34. Vite CH, Insko EK, Schotland HM, Panckeri K, Hendricks JC. Quantification of cerebral ventricular volume in english bulldogs. *Vet Radiol Ultrasound*. 1997;38(6):437-443.

35. Vullo T, Korenman E, Manzo RP, Gomez DG, Deck MD, Cahill PT. Diagnosis of cerebral ventriculomegaly in normal adult beagles using quantitative MRI. *Vet Radiol Ultrasound*. 1997;38(4):277-281.

36. Vullo T, Manzo R, Gomez DG, Deck MD, Cahill PT. A canine model of acute hydrocephalus with MR correlation. *AJNR Am J Neuroradiol*. 1998;19(6):1123-1125.

37. Yamada H, Yokota A, Haratake J, Horie A. Morphological study of experimental syringomyelia with kaolin-induced hydrocephalus in a canine model. *J Neurosurg*. 1996;84(6):999-1005. doi: 10.3171/jns.1996.84.6.0999 [doi].

38. Yamada S, Ducker TB, Perot PL. Dynamic changes of cerebrospinal fluid in upright and recumbent shunted experimental animals. *Childs Brain*. 1975;1(2-3):187-192.

39. Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden markov random field model and the expectation-maximization algorithm. *IEEE Trans Med Imaging*. 2001;20(1):45-57. doi: 10.1109/42.906424 [doi].

40. Zhao K, Sun H, Shan Y, Mao BY, Zhang H. Cerebrospinal fluid absorption disorder of arachnoid villi in a canine model of hydrocephalus. *Neurol India*. 2010;58(3):371-376.

ACKNOWLEDGMENT
We would like to thank to Attila Andics, Ádám Kettinger, Dóra Szabó, Anna Gábor for their work in the MRI experiments for Márta Gácsi for training the dogs, and Patrizia Piotti for the helpful input.