Received: 31 May 2018 | Revised: 12 June 2019 | Accepted: 9 August 2019

DOI: 10.1111/ahe.12489

ORIGINAL ARTICLE

ANATOMIA HISTOLOGIA EMBRYOLOGIA JOURNAL OF VETERINARY MEDICINE WILEY

A morphological and stereological study on brain, cerebral hemispheres and cerebellum of New Zealand rabbits

Muhammet Lütfi Selçuk[1] | **Saadettin Tıpırdamaz**[2]

[1]Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Karamanoglu Mehmetbey University, Karaman, Turkey

[2]Department of Anatomy, Faculty of Veterinary Medicine, Selcuk University, Konya, Turkey

Correspondence
Muhammet Lütfi Selçuk, Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Karamanoğlu Mehmetbey University, 70100 Karaman, Turkey.
Email: mlselcuk@hotmail.com

Abstract

The aim of this research was to determine brain, cerebral hemispheres and cerebellum volume and volume ratios by using stereological methods and investigate morphological differences between female and male New Zealand rabbits. The study was applied on 14-month old (10 male and 10 female) New Zealand rabbits. The materials removed from the cavum cranii using dorsal approach. After following routine histological procedure, paraffin blocks were prepared and cut every seventieth section at 10 μm thickness. Slides were stained with Crossmon's triple stain and photographed. The sectional images obtained were transferred to ImageJ program to estimate grey and white matter volume on cerebral hemispheres and cerebellum with principle of Cavalieri. According to results, there was no asymmetry on the left and right cerebral hemispheres of New Zealand rabbits. In the total hemisphere volume calculated by Cavalieri principle, grey and white matter ratio was 81.57% and 18.43% in female, 82.80% and 17.20% in male. It was found that the white matter was significantly higher in females than males in cerebral hemispheres ($p < .05$). Also, it was found that grey and white matter ratio in total cerebellum volume was 67.82% and 32.18% in female, 67.94% and 32.06% in male respectively. It was determined that the females' white matter was larger than male rabbits in cerebellum ($p < .05$). It is thought that morphometric data obtained from this study will contribute to the existing anatomical knowledge and also considered as reference values in the clinical sciences.

KEYWORDS
brain, Cavalieri principle, cerebellum, cerebral hemispheres, Crossmon's triple staining, stereology

1 | INTRODUCTION

The brain is subdivided into five major regions, the largest being the telencephalon, which is composed of the cerebral hemispheres; the other regions are as follows: the diencephalon, whose component parts are the epithalamus, thalamus, hypothalamus and subthalamus; the mesencephalon, consisting of the cerebral peduncles (tegmentum and crus cerebri) and the tectum (superior and inferior colliculi); the metencephalon, including the pons and cerebellum and the myelencephalon (medulla oblongata; Patestas & Gartner, 2016). When the cerebral hemispheres and cerebellum are cut, two different regions, the grey and white matter, are seen. Grey matter has nerve cells, dendrites, axon endings, unmyelinated axons and neuroglia, while white matter forms myelinated nerve extensions and is involved in nerve conduction (Bolat, 2018).

Distinct brain diseases are associated with changes in brain volume. This changes are emphasized the literature on the

Determinate brain, cerebral hemispheres and cerebellum volume and volume ratios by using stereological methods and investigate morphological differences between female and male New Zealand rabbits.

detection of brain diseases (Andersen, Andersen, & Pakkenberg, 2012; Heggland, Storkaas, Soligard, Kobro-Flatmoen, & Witter, 2015; Wang et al., 2002). The ratio of grey and white matter is of great importance to detection and in the manner of development of a disease in the search for chronic diseases affecting the central nervous system, particularly epilepsy, schizophrenia, Williams syndrome, Parkinson's disease and cortical developmental malformations due to their high prevalence, clinic symptoms and often poor prognosis for the physicians (Bas et al., 2009; Heggland et al., 2015; Selcuk & Bahar, 2014). In the study on the relationship between grey matter volume and cognitive functions in early Parkinson's disease patients without dementia, it was found that cortical grey matter volume was reduced in early Parkinson's disease compared with controls and this loss was related to Parkinson's disease cognitive status (Lee et al., 2013). Temporal lobe epilepsy studies which using continuous video-EEG (electroencephalography) monitoring have been reported to show white matter abnormalities in the connections of the frontal lobe (Riley et al., 2010; Scanlon et al., 2013). These abnormalities were also seen in the frontotemporal connections, temporooccipital connections and cerebellum (Riley et al., 2010). In studies conducted to reveal cortical anomalies associated with the genetic deletion in Williams syndrome on brain maturation, it has been reported that the cortical area of the brain is thickened (Thompson et al., 2005).

Animal models are essential for understanding brain and neurodevelopment. From primates to small animals such as rats, mice and rabbits, many species have been used in neuroscience research. Rabbits are widely used for modelling brain damage after perinatal injury in humans because the timing of perinatal brain white matter maturation is similar to human (Derrick, Drobyshevsky, Ji, & Tan, 2007; Muñoz-Moreno et al., 2013). Cerebellum, which has a complex structure, reported that not only has motor functions but also plays a role in cognitive functions in researches made in recent years. This is supported by the reason of fact that cerebellum has more than 50% of neurons in the brain (Gottwald, Wilde, Mihajlovic, & Mehdorn, 2004; Hoogendam et al., 2012). So, the determination of volume and volume ratios of grey and white matter of brain, cerebral hemispheres and cerebellum is crucial for understanding the relationship between tissue atrophy and clinical status (Gilmore et al., 2009).

MRI images have been used in recent years to examine the brain diseases in human. However, in order to find out the treatment of these diseases, studies on experimental animals are designed. Only by imaging techniques, such as MRI, are possible to estimate the volume of the internal cerebral hemisphere compartments (Cruz-Orive, 1993; Gundersen, Jensen, Kieu, & Nielsen, 1999). Special advanced devices are required to apply imaging methods in animals (Bailey et al., 2016). This increases the cost of work and reduces the applicability. In the Cavalieri's principle, the structure of interest is cut at equal intervals and the volume is estimated by calculating the area of each section. In the area of estimation, a measurement ruler is used which is named as the point counting grid and has dots located at even intervals. The biological structure of interest and the volumes of structures are estimated in an objective manner (Bolat, 2018; Gundersen et al., 1999). This method is cheaper compared with imaging methods.

The information on the morphometric properties of the brain, cerebral hemispheres and cerebellum and their differences in females and males were very limited. In the current study, it was aimed to determine volume and volume ratios of brain, cerebral hemispheres, cerebellum, grey and white matter, gender differences of New Zealand rabbits using Cavalieri principle.

2 | MATERIALS AND METHODS

2.1 | Material

Forty months old (10 male and 10 female) healthy New Zealand rabbits obtained Experimental Medicine Research and Application Center of Selcuk University were used in this study. The rabbits were housed in individual stainless steel cages in a controlled environment at a temperature of 20–25°C with 12 hr light/dark cycle per day and ad libitum water and food. The research was approved by The Ethical Committee of Experimental Medicine Research and Application Center of Selcuk University (2016/04, 2016-9).

2.2 | Methods

2.2.1 | Preparation of Cadavers

Animals were anesthetized by administration of xylazine hydrochlorure (10 mg/kg, IM) plus ketamine hydrochloride (30 mg/kg, IM; Flecknell, 2015). Abdominal cavity of the animals in the supine position was entered an incision along abdominal wall and was given 10% formalin saline into abdominal aorta. Euthanasia was carried out by an incision made on the vena cava caudalis. Cadavers were kept in 10% formalin solution for 15 days at room temperature.

After this period, the cavum cranii was opened properly and brains were removed. The brain's weight was measured. Cerebral hemispheres, medulla oblongata and cerebellum were separated from each other by a transversal section made behind rostral colliculus. The separation of the hemispheres and pons was performed on histological sections.

2.2.2 | Histological process and tissue sampling

Following routine histolological procedure, which includes dehydration in an ascending series of ethanol rinses, paraffin blocks were prepared and cut with a rotary microtome (RM 2125 RT, Leica) into 10 μm section. In scientific studies, the sampling of the biological tissue should be far from systematic bias. Each piece of the object to be sampled in the microscopic studies should have an equal effect on the result obtained by the measurement and should have the chance to be sampled equally (Cruz-Orive, 1993; Gundersen et al., 1999). Therefore, to perform systematic random sampling, a cross-section was chosen randomly among the initial 30 cross-sections, and each of the following 70th cross-sections was taken. As a result of systematic random sampling between 26 and 31, cross-sections were obtained from female and male rabbits. The sections were oven-dried at 37°C for 24 hr and stained by Crossmon's triple staining method (Crossmon, 1937). The staining protocol was given in Table 1. As a result of this staining, neurons are blue, axons are painted pale pink (Figure 1). The positive image scan option of a standard flatbed office scanner was used to obtain images (JPG, 600 DPI) for the measurements.

2.2.3 | Area and volume calculations in brain, cerebral hemispheres and cerebellum

In the volume calculations (brain, cerebral hemispheres, cerebellum, grey and white matter), grid function of the ImageJ program was used (Schneider, Rasband, & Eliceiri, 2012). ImageJ was calibrated first and point counting grid (d = 1 mm) was applied on the cross-sectional images (Figure 1). The points on grey and white matter were counted (Figure 2). The volumes were estimated as $V = a(p) \times \Sigma p \times t$ formula. In this formula, V is the volume of the structure concerned, $a(p)$ is the area of the one point on the grid (this value is 1 mm^2 in the study), Σp is the sum of the points on the structure of interest and t is the section thickness (Bahar, Bolat, & Selcuk, 2013; Gundersen et al., 1999; Howard & Reed, 2005; Selcuk & Bahar, 2014).

Particularly in volume measurements, coefficient of error (CE) is preferred smaller than or equal to 10%. In stereology studies, more than one method is used for CE calculation. In this study, the CE formula of Gundersen et al. (1999) was used. The cerebral hemispheres, pons, medulla oblongata and cerebellum ratios were calculated by dividing the related organ to the whole brain volume. In cerebellar

TABLE 1 Crossmon's triple staining procedure

Staining stages					
1	Xylene, 5 min	11	Rinse in distilled water	21	Rinse in distilled water
2	Xylene, 5 min	12	Tap water for 5 min	22	70% alcohol dip once
3	100% alcohol, 3 min	13	50% methyl alcohol, 1 min	23	80% alcohol dip once
4	100% alcohol, 3 min	14	Tap water for 5 min	24	96% alcohol dip once
5	96% alcohol, 3 min	15	Rinse in distilled water, 5 min	25	96% alcohol, 3 min
6	80% alcohol, 3 min	16	Transfer to 0.3% acid fuchsin, 0.15% orange G, 0.05% timol solution, 10 s	26	100% alcohol, 3 min
7	70% alcohol, 3 min	17	Rinse in distilled water	27	100% alcohol, 3 min
8	Rinse in distilled water	18	Transfer to 3% phosphotungstic acid, 12 min	28	Xylene, 5 min
9	Tap water for 5 min	19	Rinse in distilled water, 12 min	29	Xylene, 5 min
10	Transfer to haematoxylin solution, 10 min	20	2% anilin blue and 2% acetic acid 2% solution, 3 min	30	Xylene, 5 min

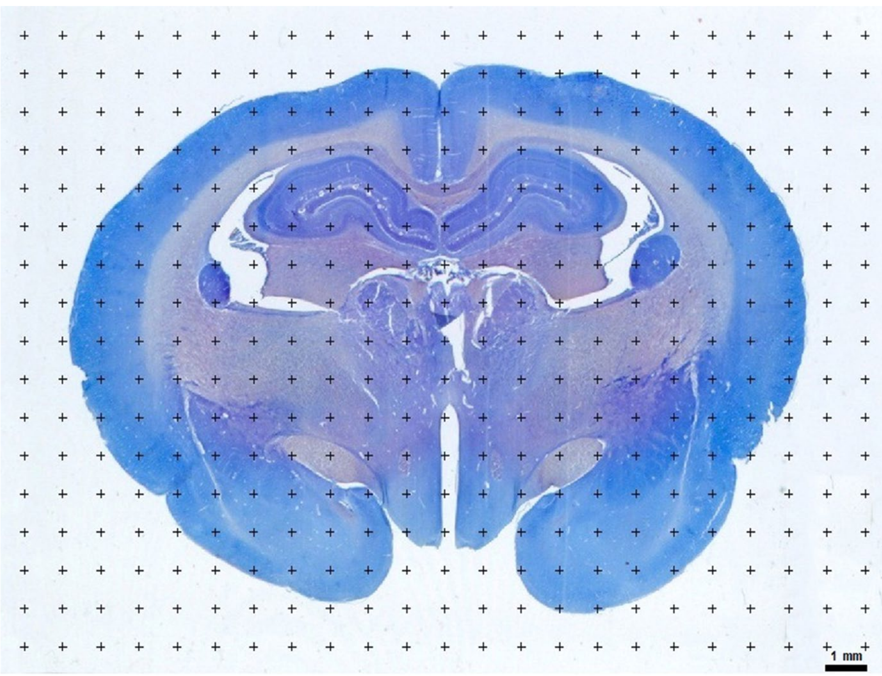

FIGURE 1 Application of point counting grid on histological sections of the brain

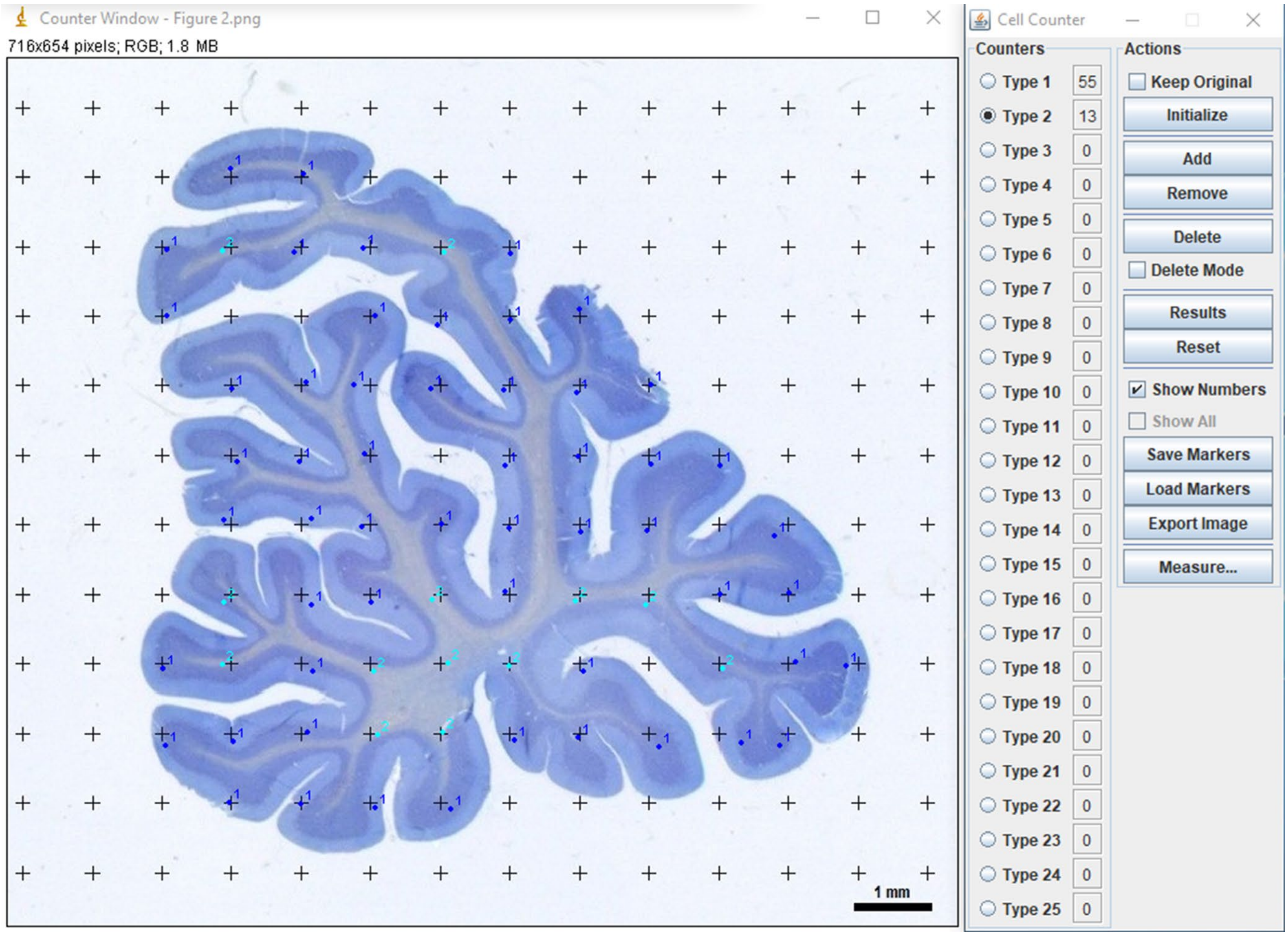

FIGURE 2 Grey and white matter counting on cerebellum with ImageJ program

hemispheres and cerebellum grey and white matter, volume ratios were obtained by dividing the volume of total related organ. Relative weight was obtained by dividing the weight of the structure of interest by the weight of the whole body.

2.3 | Statistical analysis

Grey matter, white matter and hemisphere volumes were compared by Paired sample *t* test. Statistical analysis was performed using the GraphPad Prism 6 for Windows. $p < .05$ was accepted statistically significant. Data are expressed as means ± standard error (*SE*).

3 | RESULTS

As a result of measurements, the mean weight of female and male New Zealand rabbits used was 3,264 ± 152 and 2,662 ± 86 g, brain weights were 9.98 ± 0.22 and 9.89 ± 0.20 g, cerebellum weights were 1.57 ± 0.05 and 1.53 ± 0.03 g respectively. The brain volume was 9.77 ± 0.20 ml in females and 9.78 ± 0.23 ml in males. The cerebellum volume was 0.87 ± 0.01 ml in females and 0.78 ± 0.03 ml in males (Table 2). Cerebral hemisphere and pons, medulla oblongata and cerebellum ratio were 78.81%, 5.94% and 15.25% in female, 78.02%, 6.54% and 15.44% in male respectively. Relative weights of the brain and cerebellum were 0.31 ± 0.1 and 0.048 ± 0.002 in female, 0.37 ± 0.01 and 0.057 ± 0.001 in male (Table 2).

TABLE 2 The body, organ weights and volume of the animals used in the study (Mean ± *SE*)

Parameters	Female	Male
Body weight (g)	3,264 ± 152	2,662 ± 86
Brain		
Weight (g)	9.98 ± 0.22	9.89 ± 0.20
Volume (ml)	9.77 ± 0.20	9.78 ± 0.23
Cerebellum		
Weight (g)	1.57 ± 0.05	1.53 ± 0.03
Volume (ml)	0.87 ± 0.01	0.78 ± 0.03
Relative weight		
Brain	0.31 ± 0.1	0.37 ± 0.01
Cerebellum	0.048 ± 0.002	0.057 ± 0.001

TABLE 3 The morphometric data of the cerebral hemispheres (Mean ± *SE*)

Animal gender	Total cerebral hemisphere volume (ml)	Grey matter volume (ml)	CE value	Grey matter ratio (%)	White matter volume (ml)	CE value	White matter ratio (%)
Female	2.66 ± 0.17	2.17 ± 0.14	0.017 ± 0.001	81.57	0.49 ± 0.08	0.016 ± 0.0006	18.43
Male	2.50 ± 0.19	2.07 ± 0.17	0.027 ± 0.001	82.80	0.43 ± 0.05	0.022 ± 0.001	17.20
p	.0675	.2004	–	–	.0289*	–	–

*$p < .05$.

The mean values of grey and white matter volume in females and males calculated with Cavalieri principle were given in Table 3. It was found that the white matter was significantly higher in females than males in cerebral hemispheres and it was statistically significant ($p < .05$). No difference in grey matter between male and female rabbits was detected ($p > .05$).

In the female New Zealand rabbits, the percentages of the left and right cerebral hemispheres grey and white matter ratios were found to be 81.81%, 18.19%, 81.34% and 18.66%, respectively. In male New Zealand rabbits, these ratios were 82.92%, 17.08% and 82.67%, 17.33%. It was found that the left and right white matter was significantly higher in females than males in cerebral hemispheres ($p < .05$; Table 4).

No asymmetry was found between the right and left cerebral hemispheres in females and males. Similarly, there was no asymmetry between the grey and white matter of the right and left cerebral hemispheres in female and male rabbits ($p > .05$).

Morphometric data of cerebellum were given in Table 5. In female and male New Zealand rabbits, the average volume of the cerebellums was 0.87 ± 0.01 and 0.78 ± 0.03 ml, respectively. The average volume of cerebellum constituted 67.82% grey matter and 32.18% white matter in female New Zealand rabbits. This rate was 67.94% grey matter and 32.06% white matter in male New Zealand rabbits. It was found that while the total cerebellum volume and white matter were significantly higher in females than males ($p < .05$), there was no difference grey matter ($p > .05$).

As a result of estimation made on cerebellum, there was a significant difference between female and male total cerebellum and white matter volume averages, and the cerebellum in females was larger than males. It was also determined that the females white matter was larger than male rabbits ($p < .05$).

4 | DISCUSSION

Nervous tissue contains different structures such as neurons and glial cells. An accurate characterisation of their complex arrangement in healthy tissue can help in the understanding of neurological diseases. Animal models play an important role in the development of neuroscience, and many models have been established to investigate neurocognitive diseases (Eixarch et al., 2012; Ferraris et al., 2018). However, translation values are limited due to the slow myelination of rodents in prenatal brain development, lysencephalic brain structures and low white matter ratio (Coelho et al., 2018; Ferraris et al., 2018). Unlike all rodents, rabbits are similar to people in perinatal brain white matter maturation time (Eixarch et al., 2012). Considering costly models used for perinatal brain injury, it is more attractive to use rabbit models (Coelho et al., 2018). The focus of this work was to characterise volume fraction distribution of brain, cerebral hemispheres, cerebellum, grey and white matter and gender differences in New Zelland Rabbits, which has not been previously addressed in the literature.

TABLE 4 Morphometric data of right and left cerebral hemisphere (Mean ± *SE*)

Animal gender	Left cerebral hemisphere (ml)	Grey matter (ml)	White matter (ml)	Right cerebral hemisphere (ml)	Grey matter (ml)	White matter (ml)
Female	1.32 ± 0.11	1.08 ± 0.03	0.24 ± 0.01	1.34 ± 0.07	1.09 ± 0.001	0.25 ± 0.01
Male	1.23 ± 0.10	1.02 ± 0.02	0.21 ± 0.01	1.27 ± 0.09	1.05 ± 0.02	0.22 ± 0.01
p	.1117	.2217	.0398*	.0897	.2348	.0312*

*$p < .05$.

TABLE 5 Morphometric data of cerebellum (Mean ± *SE*)

Animal gender	Total cerebellum volume (ml)	Grey matter (ml)	White matter (ml)	CE value
Female	0.87 ± 0.01	0.59 ± 0.01	0.28 ± 0.01	0.024 ± 0.0008
Male	0.78 ± 0.03	0.53 ± 0.02	0.25 ± 0.009	0.026 ± 0.002
p	.0469*	.0858	.0499*	–

*$p < .05$.

Kolb, Sutherland, Nonneman, and Whishaw (1982) found asymmetry in the asymmetry study of rats, cat and rabbits, reported that the right cerebral hemisphere was larger than the left cerebral hemisphere. Asymmetry was not detected during the study conducted by Mayhew, Mwamengele, and Dantzer (1996) on horse, cattle, pig, goat, dog, cat and rabbit brain. Barbeito-Andrés, Bernal, and Gonzalez (2016) found fluctuating asymmetry in mouse brain. Heilbroner and Holloway (1989) reported that cerebral hemispheres exhibit symmetry in their work on female and male ape brains. Henery and Mayhew (1989) found symmetry in the human brain. Cuce et al. (2014) found symmetry in children aged 1–5 years too. In the presented study, asymmetry was not detected on the left and right cerebral hemisphere volumes. It was thought that the current differences were caused by the animal species, age range and method used.

Mayhew et al. (1996) found that brain volume in the rabbit was 7 ± 0.57 ml. It was reported that grey matter is larger in smaller mammals and smaller in larger animals. In present study, brain volume was found to be 9.77 ± 0.20 and 9.78 ± 0.23 ml in females and males respectively. Grey matter was found to be greater in females and males, and two studies showed compliance.

A study using brain MRI images of the rabbit, it was found that the central nervous system was consist of 81.32% hemisphere and pons, 4.13% medulla oblongata and 14.55% cerebellum. The cerebral hemispheres were composed of 90.22% grey matter, and 9.28% white matter (Muñoz-Moreno et al., 2013). In present study, hemisphere and pons, medulla oblongata and cerebellum ratio were 78.81%, 5.94% and 15.25% in female, 78.02%, 6.54% and 15.44% in male respectively. Grey and white matter ratios of cerebral hemispheres were 81.57%, 18.43% in female and 82.80%, 17.20% in male. In MRI images, the substantia grisea and alba border cannot be clearly separated (Selcuk & Bahar, 2014). It is thought that the difference between the two studies was due to the methodological difference and the absence of sexual dimorphism on MRI study.

In a study in which no sexual dimorphism was performed by Karabekir et al. (2014) on seven rabbits, cerebellum volume was reported as 0.69 ± 0.03 ml. In present study, cerebellum volume was determined as 0.87 ± 0.01 ml in female rabbits and 0.78 ± 0.03 ml in male rabbits. It was thought that the difference between the two studies could be caused by the age difference of the animals used and the lack of no sexual dimorphism in the study made by Karabekir et al. (2014).

It is reported that the 10% or less of the CE in volume calculations made with the Cavalieri principle is an important parameter in terms of reliability of the study (García-Fiñana, Cruz-Orive, Mackay, Pakkenberg, & Roberts, 2003; Gundersen & Jensen, 1987; Gundersen et al., 1999). In the study, this value was calculated separately for brain, cerebellum, grey and white matter and found that the values obtained were reliable.

The focus of this work was to characterise volume fraction of brain, cerebral hemispheres, cerebellum, grey and white matter by doing sexual dimorphism, which has not been previously addressed in the literature in New Zealand Rabbits. It is thought that these morphometric data that are given with tables would be very helpful in diagnosis of brain and cerebellum diseases and treatment. Stereological methods in determination of brain morphometry in New Zealand rabbits were successfully implemented for the first time in this study, and this will contribute significantly to the future studies.

ACKNOWLEDGEMENTS

This research was summarized from the researchers PhD. thesis. The research abstract was presented and published in I. International Veterinary Congress of Turkey, Sandıklı/Afyonkarahisar 2017.

CONFLICT OF INTEREST

The authors have no conflict of interest. The authors alone are responsible for the content and writing of paper.

ORCID

Muhammet Lütfi Selçuk https://orcid.org/0000-0002-9915-3829

Saadettin Tıpırdamaz https://orcid.org/0000-0003-4786-2612

REFERENCES

Andersen, K., Andersen, B. B., & Pakkenberg, B. (2012). Stereological quantification of the cerebellum in patients with Alzheimer's disease. *Neurobiology of Aging*, *33*(1), 11–20. https://doi.org/10.1016/j.neurobiolaging.2010.06.013

Bahar, S., Bolat, D., & Selcuk, M. L. (2013). The segmental morphometric properties of the horse cervical spinal cord: A study of cadaver. *The Scientific World Journal*, *2013*, 1–9. https://doi.org/10.1155/2013/734923

Bailey, D. L., Pichler, B. J., Gückel, B., Barthel, H., Beer, A. J., Botnar, R., … Lanzenberger, R. (2016). Combined PET/MRI: From status quo to status go. Summary report of the fifth international workshop on PET/MR imaging; February 15–19, 2016; Tübingen, Germany. *Molecular Imaging and Biology*, *18*(5), 637–650.

Barbeito-Andrés, J., Bernal, V., & Gonzalez, P. N. (2016). Morphological asymmetries of mouse brain assessed by geometric morphometric analysis of MRI data. *Magnetic Resonance Imaging*, *34*(7), 980–989. https://doi.org/10.1016/j.mri.2016.04.006

Bas, O., Acer, N., Mas, N., Karabekir, H. S., Kusbeci, O. Y., & Sahin, B. (2009). Stereological evaluation of the volume and volume fraction of intracranial structures in magnetic resonance images of patients with Alzheimer's disease. *Annals of Anatomy-Anatomischer Anzeiger*, *191*(2), 186–195. https://doi.org/10.1016/j.aanat.2008.12.003

Bolat, D. (2018). Estimation of volume of ox brain and gray and white matter with Cavalier's principle. *Kocatepe Veterinary Journal*, *11*(1), 30–34.

Coelho, S., Pozo, J. M., Costantini, M., Highley, J. R., Mozumder, M., Simpson, J. E., … Frangi, A. F. (2018). Local volume fraction distributions of axons, astrocytes, and myelin in deep subcortical white matter. *NeuroImage*, *179*, 275–287. https://doi.org/10.1016/j.neuroimage.2018.06.040

Crossmon, G. (1937). A modification of Mallory's connective tissue stain with a discussion of the principles involved. *The Anatomical Record*, *69*, 33–38. https://doi.org/10.1002/ar.1090690105

Cruz-Orive, L. M. (1993). Systematic sampling in stereology. *Bulletin of the International Statistical Institute*, *2*, 451–468.

Cuce, G., Seker, M., Kalkan, S., Canbilen, A., Sakarya, M. E., Cuce, H., & Ozbek, O. (2014). Evaluation of cerebrum volume of children 1–5 years old. *Biomedical Research*, *25*(4), 603–605.

Derrick, M., Drobyshevsky, A., Ji, X., & Tan, S. (2007). A model of cerebral palsy from fetal hypoxia-ischemia. *Stroke*, *38*(2), 731–735. https://doi.org/10.1161/01.STR.0000251445.94697.64

Eixarch, E., Batalle, D., Illa, M., Muñoz-Moreno, E., Arbat-Plana, A., Amat-Roldan, I., … Gratacos, E. (2012). Neonatal neurobehavior and diffusion MRI changes in brain reorganization due to intrauterine growth restriction in a rabbit model. *PLoS ONE*, *7*(2), 187–198. https://doi.org/10.1371/journal.pone.0031497

Ferraris, S., van der Merwe, J., Van Der Veeken, L., Prados, F., Iglesias, J.-E., Melbourne, A., … Vercauteren, T. (2018). A magnetic resonance multi-atlas for the neonatal rabbit brain. *NeuroImage*, *179*, 187–198. https://doi.org/10.1016/j.neuroimage.2018.06.029

Flecknell, P. (2015). *Laboratory animal anaesthesia*, 4th ed. Oxford, UK: Academic Press.

García-Fiñana, M., Cruz-Orive, L. M., Mackay, C. E., Pakkenberg, B., & Roberts, N. (2003). Comparison of MR imaging against physical sectioning to estimate the volume of human cerebral compartments. *NeuroImage*, *18*(2), 505–516. https://doi.org/10.1016/S1053-8119(02)00021-6

Gilmore, C. P., Donaldson, I., Bö, L., Owens, T., Lowe, J., & Evangelou, N. (2009). Regional variations in the extent and pattern of grey matter demyelination in multiple sclerosis: A comparison between the cerebral cortex, cerebellar cortex, deep grey matter nuclei and the spinal cord. *Journal of Neurology, Neurosurgery & Psychiatry*, *80*(2), 182–187. https://doi.org/10.1136/jnnp.2008.148767

Gottwald, B., Wilde, B., Mihajlovic, Z., & Mehdorn, H. (2004). Evidence for distinct cognitive deficits after focal cerebellar lesions. *Journal of Neurology, Neurosurgery & Psychiatry*, *75*(11), 1524–1531. https://doi.org/10.1136/jnnp.2003.018093

Gundersen, H. J. G., & Jensen, E. (1987). The efficiency of systematic sampling in stereology and its prediction. *Journal of Microscopy*, *147*(3), 229–263. https://doi.org/10.1111/j.1365-2818.1987.tb02837.x

Gundersen, H. J. G., Jensen, E. B. V., Kieu, K., & Nielsen, J. (1999). The efficiency of systematic sampling in stereology reconsidered. *Journal of Microscopy*, *193*(3), 199–211. https://doi.org/10.1046/j.1365-2818.1999.00457.x

Heggland, I., Storkaas, I. S., Soligard, H. T., Kobro-Flatmoen, A., & Witter, M. P. (2015). Stereological estimation of neuron number and plaque load in the hippocampal region of a transgenic rat model of Alzheimer's disease. *European Journal of Neuroscience*, *41*(9), 1245–1262. https://doi.org/10.1111/ejn.12876

Heilbroner, P. L., & Holloway, R. L. (1989). Anatomical brain asymmetry in monkeys: Frontal, temporoparietal, and limbic cortex in Macaca. *American Journal of Physical Anthropology*, *80*(2), 203–211. https://doi.org/10.1002/ajpa.1330800208

Henery, C. C., & Mayhew, T. M. (1989). The cerebrum and cerebellum of the fixed human brain: Efficient and unbiased estimates of volumes and cortical surface areas. *Journal of Anatomy*, *167*, 167.

Hoogendam, Y. Y., van der Geest, J. N., van der Lijn, F., van der Lugt, A., Niessen, W. J., Krestin, G. P., … Ikram, M. A. (2012). Determinants of cerebellar and cerebral volume in the general elderly population. *Neurobiology of Aging*, *33*(12), 2774–2781. https://doi.org/10.1016/j.neurobiolaging.2012.02.012

Howard, V., & Reed, M. (2005). *Unbiased stereology: Three-dimensional measurement in microscopy*, 2nd ed. Oxford, UK: Garland Science/Bios Scientific Publishers.

Karabekir, H. S., Akosman, M. S., Gocmen-Mas, N., Aksu, F., Edizer, M., Lenger, O. F., & Turkmenoglu, I. (2014). The volume of experimental design cerebellum: Stereological microanatomic study. *Journal of Craniofacial Surgery*, *25*(4), 1492–1494. https://doi.org/10.1097/SCS.0000000000000845

Kolb, B., Sutherland, R. J., Nonneman, A. J., & Whishaw, I. Q. (1982). Asymmetry in the cerebral hemispheres of the rat, mouse, rabbit, and cat: The right hemisphere is larger. *Experimental Neurology*, *78*(2), 348–359. https://doi.org/10.1016/0014-4886(82)90053-X

Lee, E.-Y., Sen, S., Eslinger, P. J., Wagner, D., Shaffer, M. L., Kong, L., … Huang, X. (2013). Early cortical gray matter loss and cognitive correlates in non-demented Parkinson's patients. *Parkinsonism & Related Disorders*, *19*(12), 1088–1093. https://doi.org/10.1016/j.parkreldis.2013.07.018

Mayhew, T., Mwamengele, G., & Dantzer, V. (1996). Stereological and allometric studies on mammalian cerebral cortex with implications for medical brain imaging. *Journal of Anatomy*, *189*(Pt 1), 177.

Muñoz-Moreno, E., Arbat-Plana, A., Batalle, D., Soria, G., Illa, M., Prats-Galino, A., … Gratacos, E. (2013). A magnetic resonance image based atlas of the rabbit brain for automatic parcellation. *PLoS ONE*, *8*(7), 1–13. https://doi.org/10.1371/journal.pone.0067418

Patestas, M. A., & Gartner, L. P. (2016). *A textbook of neuroanatomy* (pp. 5–9). Malden, MA: Blackwell Publishing.

Riley, J. D., Franklin, D. L., Choi, V., Kim, R. C., Binder, D. K., Cramer, S. C., & Lin, J. J. (2010). Altered white matter integrity in temporal lobe epilepsy: Association with cognitive and clinical profiles. *Epilepsia*, *51*(4), 536–545. https://doi.org/10.1111/j.1528-1167.2009.02508.x

Scanlon, C., Mueller, S. G., Cheong, I., Hartig, M., Weiner, M. W., & Laxer, K. D. (2013). Grey and white matter abnormalities in temporal lobe epilepsy with and without mesial temporal sclerosis. *Journal of Neurology*, *260*(9), 2320–2329. https://doi.org/10.1007/s00415-013-6974-3

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Selcuk, M. L., & Bahar, S. (2014). The morphometric properties of lumbar spinal cord segments in horses. *Journal of Animal and Veterinary Advances*, *13*(11), 653–659.

Thompson, P. M., Lee, A. D., Dutton, R. A., Geaga, J. A., Hayashi, K. M., Eckert, M. A., … Toga, A. W. (2005). Abnormal cortical complexity and thickness profiles mapped in Williams syndrome. *Journal of Neuroscience*, *25*(16), 4146–4158. https://doi.org/10.1523/JNEUROSCI.0165-05.2005

Wang, D., Chalk, J. B., Rose, S. E., de Zubicaray, G., Cowin, G., Galloway, G. J., … Semple, J. (2002). MR image-based measurement of rates of change in volumes of brain structures. Part II: Application to a study of Alzheimer's disease and normal aging. *Magnetic Resonance Imaging*, *20*(1), 41–48. https://doi.org/10.1016/S0730-725X(02)00472-1

How to cite this article: Selçuk ML, Tıpırdamaz S. A morphological and stereological study on brain, cerebral hemispheres and cerebellum of New Zealand rabbits. *Anat Histol Embryol*. 2020;49:90–96. https://doi.org/10.1111/ahe.12489