The Volume of Experimental Design Cerebellum: Stereological Microanatomic Study

H. Selim Karabekir, MD, M. Sirri Akosman, PhD,† Nuket Gocmen-Mas, MD, PhD,‡ Funda Aksu, MD,‡ Mete Edizer, MD, PhD,‡ Omer F. Lenger, PhD,§ and Ismail Turkmenoglu, PhD†*

Abstract: Cerebellum is responsible to higher cognitive functions and delicate motor activity. Because of its significance, cerebellum may play a major role in cognitive, behavioral, and psychiatric diseases. Therefore, the investigations on cerebellum may be contributed to the future researches so as to solve reasons of the diseases. The rabbit brain relatively larger than the rat and mouse brain and the individual evaluation of its subdivisions are rather easy. There are no data on cerebellar volume of rabbits in literature. Thus, the study presents microscopic volume calculation of rabbits' cerebellum using the stereological method. The mean (SD) calculated volume of the cerebellum in the rabbit brain was found to be 0.69 (0.03) cm^3. The individual microscopic volume estimation of an animal subject can be achieved by Cavalier method. Researchers believed that the findings and the applied method in this study may be useful for the scientists.

Key Words: Cerebellum, rabbit, rat, volume, cell calculation

(*J Craniofac Surg* 2014;25: 1492–1494)

Microscopically, the cerebellar cortex is formed by granule cells, Purkinje cells, Golgi cells, 2 types of inhibitory interneurones, and stellate cells.[1] Three kinds of excitator extracerebellar afferents climb to the cortex such as the climbing and mossy fibers.[2] In literature, several investigations were declared about the cellular anatomy of the cerebellum on animal models.[3] The function of the cerebellum has been seemed as limited to the coordination of posture, voluntary movement, gait, speech, and delicate motor functions. Addionally, the cerebellum may have a mission in cognitive, behavioral, and psychiatrical diseases.[4–6] Cerebellum also have a role on working memory function.[7]

Higher cognitive functions continue to develop during childishness and puberty in human. As exactly alike, the cerebellum also develops from childishness to puberty and accesses the peak levels between 10 and 13 years old for both sexes.[8] In the current study, we aimed to calculate the volume of the rabbit cerebellum via stereological method to further contribute to experimental studies.

MATERIALS AND METHODS

This study was performed for the total cerebellar volume on brains of 7 rabbits (1–1.5 y, 3–4 kg). The housing and caring of the animals, breeding and whelping, brain dissections, perfusion, and euthanasia were approved by the respective Institutional Animal Care and Use Committees of the institutions and were designed according to the principles described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals were anesthetized with isoflurane and decapitated. Scalp skin of rats was incised under anesthesia from the median line. The soft tissue on the upper part of the calvary was retracted, and the skull dissected from the midline reached the brain. Brains were quickly removed from the cranial cavity. The cerebellum was separated from the cerebrum and the brain tissues. The brain substructures attentively dissected under ×4 loop microscope; the cerebellum was fixed in neutral buffered 10% of formalin for 2 weeks. After immersion fixation, the cerebellum was weighed and totally embedded to the paraffin. Each cerebellum was divided from the midline of the vermis to obtain 2 equal parts as the left and right sides. One of these sides (one half) was selected randomly, the selected slice of the cerebellum was again divided from the middle, and 1 slice (one half) was again selected randomly. Again, that selected slice was sliced from the middle, and 1 slice (one half) was selected randomly. That selected slice was embedded in paraffin and was totally sliced at 40-μm thicknesses by rotary microtome (Leica RM2155), and every seventh slab was put onto a glass slide and stained by Giemsa (Giemsa azur eosin methylene blue solution; Merck) stain, as described in the previous study of our study group.[3]

All glass slides were fixed on a platform per specimen, and the area of the cerebellar tissue on the slides was measured by a 0.1-cm transparent point grid (Fig. 1). All measurements were performed blinded to subject details and data of any other results.

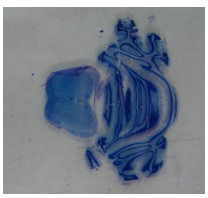


FIGURE 1. Measurement of the area of the slice obtained from a rabbit cerebellum by using point grid.

From the *Department of Neurosurgery, Faculty of Medicine, Dokuz Eylul University, Izmir; †Department of Anatomy, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar; ‡Department of Anatomy, Faculty of Medicine, Dokuz Eylul University, Izmir; and §Department of Medical Biology and Genetics, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey.

Received January 14, 2014.
Accepted for publication February 1, 2014.

Address correspondence and reprint requests to Funda Aksu, MD, Department of Anatomy, Medical Faculty, Dokuz Eylül University, 35340 Inciralti, Izmir, Turkey; E-mail: funda.aksu@deu.edu.tr

Presented as a poster presentation in the Sixth International Balkan Animal Conference, October 2013, Tekirdag, Turkey.
All authors assume responsibility for researching, calculating and evaluating while writing stages of the article.
The authors report no conflicts of interest.

Copyright © 2014 by Mutaz B. Habal, MD

ISSN: 1049-2275
DOI: 10.1097/SCS.0000000000000845

Copyright © 2014 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.

The volume of the cerebellum was calculated by the formula below for each subject:

$$V = (t \times a/p \times \sum P)\text{cm}^3$$

where t is the section thickness (0.052 cm), a/p is the area represented by a point in the grid (0.1 cm × 0.1 cm), and $\sum P$ is the total number of points hitting the surface area of sections.

The coefficient of error of the study was calculated according to Nyengaard[9] (1999).

RESULTS

All cerebellums were in normal anatomic appearance (Fig. 2). The cerebellar volumes of the rabbits were shown in the Table 1.

TABLE 1. Volume Parameters of the Cerebellum of 7 Rabbits

Cerebellum Number	Volume, cm^3
1	0.7
2	0.8
3	0.8
4	0.5
5	0.7
6	0.5
7	0.8
Mean (SD)	0.69 (0.03)

DISCUSSION

Experimental researches on the animal cerebellum aimed to find out the role of cerebellar changes on cognitive, behavioral, and psychiatric diseases. The recent researches show that the cerebellum has an important role on many diseases. The volume and neuronal number in the cerebellum can change in patients with epilepsy. Cerebellar asymmetric enlargement was declerated in intractable epilepsy as 46.7%.[10] Because of these reasons, volumetric prediction on the cerebellum is vulnerable as much as researches of the cerebellum volume in epilepsy, schizophrenia, Alzheimer disease, and so on.[10–12] The volume prediction of the cerebellum might be accurate, unbiased, efficient, cheap, and simple by using cavalier method. Akosman et al[13] (2013) and Gocmen-Mas et al[14] (2013) used the mentioned method to calculate volumes of the rabbits' thalamus and hippocampus.

Although several experimental microscopic studies on cerebellar Purkinje cells were performed by many authors, there are no studies on estimation on the rabbits' cerebellar volume in the literature.[3,15] Paradiso et al[16] (1997) claimed that cerebellar volume correlated with general intelligence and cerebellar size has been detected to be weakly associated with memory retention. Woodruff-Pak et al (2001) reported that lower cerebellar volume in humans is related with slower conditioned reply acquisition in young adults. Wegiel et al (1999) declerated that the mean volume of Purkinje cells in the cerebellum was 25% less in the patients with autistism than in the healthy subjects. Schutter et al[17] (2012) claimed that cerebellar volume covaries with neurotic personality traits in healthy subjects and also that the cerebellum may play a role in patients with negative affect and developed mood disorders. Several authors showed that cerebellar volume decreased in patients with Alzheimer disease[11] and also in patients with cerebellar atrophy using the semiautomated volumetric technique.[18] Identified changes were associated with an overall volumetric reduction in granular and molecular layers of the cerebellum and also a reduction in the total number of Purkinje cells as contrary to controls.[19]

Right-greater-than-left cerebellar volume asymmetry and relatively larger volumes of the right hemisphere and vermian lobules such as declive, folium, and tuber were determined on human cerebellum.[20] The other study claimed that basketball players had relatively larger volumes of vermian lobules in the cerebellum than the others.[20] Schmitz and Hof[21] (2007) claimed that stereology may allow the evaluation of the global and local features of biologic architecture, the most important of which are volume, number, connectivity, spatial distribution, and length of the structures. For instance, some investigators want to display whole hippocampal volume so as to evaluate changes between the ventral and posterior parts on some diseases.[22,23]

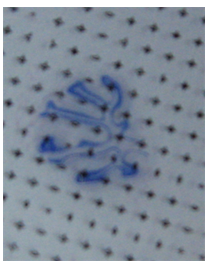


FIGURE 2. A slice from the rabbit cerebellum.

REFERENCES

1. Greenstein B, Greenstein A. *Color atlas of neuroscience*. Verlag, Germany: Georg Thieme; 2000
2. Voogd J, Glickstein M. The anatomy of the cerebellum. *Trends Cogn Sci* 1998;2:307–313
3. Akosman MS, Gocmen-Mas N, Karabekir HS. Estimation of Purkinje cell quantification and volumetry in the cerebellum using a stereological technique. *Folia Morphol* 2011;70:240–244
4. Rapoport M, van Reekum R, Mayberg H. The role of the cerebellum in cognition and behavior: a selective review. *J Neuropsychiatry Clin Neurosci* 2000;12:193–198
5. Gottwald B, Wilde B, Mihajlovic Z, et al. Evidence for distinct cognitive deficits after focal cerebellar lesions. *J Neurol Neurosurg Psychiatry* 2004;75:1524–1531
6. Hopyan T, Laughlin S, Dennis M. Emotions and their cognitive control in children with cerebellar tumors. *J Int Neuropsychol Soc* 2010;16:1027–1038
7. Ding H, Qin W, Jiang T, et al. Volumetric variation in subregions of the cerebellum correlates with working memory performance. *Neurosci Lett* 2012;508:47–51
8. Kosar MI, Karacan K, Otag I, et al. Determination of cerebellar volume in children and adolescents with magnetic resonance images. *Folia Morphol (Warsz)* 2012;71:65–70
9. Nyengaard JR. Stereologic methods and their application in kidney research. *J Am Soc Nephrol* 1999;10:1100–1123
10. Sato N, Yagishita A, Oba H, et al. Hemimegalencephaly: a study of abnormalities occurring outside the ınvolved hemisphere. *AJNR Am J Neuroradiol* 2007;28:678–682
11. Wegiel J, Wisniewski HM, Dziewiatkowski J, et al. Cerebellar atrophy in Alzheimer's disease—clinicopathological correlations. *Brain Res* 1999;818:41–50
12. Andersen BB, Pakkenberg B. Stereological quantitation in cerebella from people with schizophrenia. *Br J Psychiatry* 2003;82:354–361
13. Akosman MS, Karabekir HS, Gocmen-Mas NN, et al. Volume estimation of the rabbit thalamus: stereological microanatomical study. *Neurosciences* 2013;18:391–392

© 2014 Mutaz B. Habal, MD

Copyright © 2014 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.

14. Gocmen-Mas N, Akosman MS, Karabekir S. The Volume Prediction on the Experimental Design Hippocampus Using Stereological Method. *J Craniofac Surg* 2014;25:1510–1512
15. Woodruff-Pak DS, Vogel RW, Ewers M, et al. MRI-assessed volume of cerebellum correlates with associative learning. *Neurobiol Learn Mem* 2001;76:342–357
16. Paradiso S, Andreasen NC, O'Leary DS, et al. Cerebellar size and cognition: correlations with IQ, verbal memory and motor dexterity. *Neuropsychiatry Neuropsychol Behav Neurol* 1997;10:1–8
17. Schutter DJ, Koolschijn PC, Peper JS, et al. The cerebellum link to neuroticism: a volumetric MRI association study in healthy volunteers. *PLoS One* 2012;7:e37252
18. Hayashi N, Sanada S, Suzuki M, et al. Semiautomated volumetry of the cerebrum, cerebellum-brain stem, and temporal lobe on brain magnetic resonance images. *Radiat Med* 2008;26:104–114
19. Mrzilková J, Zach P, Bartoš A, et al. Volumetric analysis of the pons, cerebellum and hippocampi in patients with Alzheimer's disease. *Dement Geriatr Cogn Disord* 2012;34:224–234
20. Park IS, Lee KJ, Han JW, et al. Experience-dependent plasticity of cerebellar vermis in basketball players. *Cerebellum* 2009;8:334–339
21. Schmitz C, Hof PR. Design-based stereology in brain aging research. In: Riddle DR, ed. *Brain Aging: Models, Methods, and Mechanisms*. Boca Raton: CRC Press, 2007
22. Willard SL, Friedman DP, Henkel CK, et al. Anterior hippocampal volume is reduced in behaviorally depressed female cynomolgus macaques. *Psychoneuroendocrinology* 2009;34:1469–1475
23. Schmitt A, Steyskal C, Bernstein HG, et al. Stereologic investigation of the posterior part of the hippocampus in schizophrenia. *Acta Neuropathol* 2009;117:395–407

© 2014 Mutaz B. Habal, MD

Copyright © 2014 Mutaz B. Habal, MD. Unauthorized reproduction of this article is prohibited.