The Volume Prediction on the Experimental Design Hippocampus Using Stereological Method

Nuket Gocmen-Mas, MD, PhD, Murat Sirri Akosman, PhD, Vet,† Selim Karabekir, MD,‡ Ahmet Turan Isik, MD,§ M. Refik Mas, MD,§ and Funda Aksu, MD**

Downloaded from http://journals.lww.com/jcraniofacialsurgery by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIH04XMI0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 06/30/2024

Abstract: The application of stereologic techniques to the analysis of the nervous system has greatly contributed to the evaluation of the normal and pathological anatomy of the aging brain. Currently, the hippocampus still holds secrets about the aging process. Experimental researches on hippocampus morphology may contribute to the future researches. This study presents the volume and weight of left hippocampus using a stereological technique on light microscope. The mean weight of the encephalon without cerebellum was 6.1 ± 0.1 g. The mean weight and the volume of the hippocampus were (mean ± SD) 0.28 ± 0.02 g and 0.28 ± 0.02 cm^3, respectively. The mean coefficient of error for the stereological volume estimation of the hippocampus was 0.03. The individual volume estimation of the subjects may be achieved by the Cavalieri method. Investigators believed that the findings and the applied technique in this study may be useful for clinicians.

Key Words: Cavalieri method, stereology, rabbit, hippocampus, volume

(*J Craniofac Surg* 2014;25: 1501–1503)

The hippocampus is a brain structure and is located in the medial of the temporal region of the lateral ventricle. It has 3 subareas named CA1, CA2, and CA3.[1,2] Hippocampus, dentate gyrus, and subiculum together form the hippocampal formation.[3,4] Several researchers want to determine the whole hippocampal volume size so as to evaluate differences between the ventral and posterior hippocampus on some pathologies.[5–7] The Cavalieri method provides unbiased, accurate, effective, simple, and cheap volume estimations of the object.[8,9] The areas of the sections could be estimated by the regularly spaced points in a known distance transparent test probe superposed. For estimates of the areal fraction of profile, all points that hit the profile were counted.[8,9]

The study aimed to estimate the volume of the rabbit hippocampus to contribute and facilitate future experimental researches.

MATERIALS AND METHODS

Animals and Process

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 NIH Guide for the Care and Use of Laboratory Animals. All experimental procedures were approved by the Ethics Commission of Afyon Kocatepe University in 2010.

The study was performed on the left hippocampuses of 7 rabbits (1–1.5 years, 3–4 kg). The animals were anesthetized with isoflurane. 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 to the brain. Brains were quickly removed from the cranial cavity. Formalin was delivered to the lateral ventricles through the septum telencephali (pellucidum), and the tissue was left in the same formalin solution. The hippocampus was attentively dissected under 4× loop microscope, and the specimens fixed in neutral buffered 10% formalin for 2 days. Each of the hippocampus was measured before and after formalin fixation.

Sampling of the Hippocampus

After immersion fixation, to reach the individual hippocampus through the septum telencephali (pellucidum), it was totally extracted gently and attentively using ×4 loop microscope avoiding tissue damage. The hippocampus was weighed, totally embedded in paraffin, longitudinally sectioned at 40-μm thickness using a rotary microtome (Leica RM2155), and every 13th slab chosen and stained by Giemsa

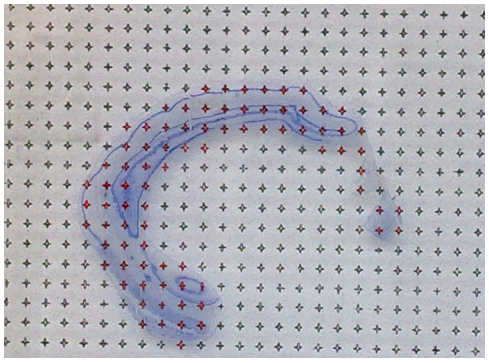


FIGURE 1. Measurement of the area of the slice obtained from the healthy rabbit hippocampus using the stereological technique.

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

Received October 9, 2013.
Accepted for publication January 7, 2014.

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

The contribution of the first two authors into the work was equal and higher than the rest of the authors.

The authors report no conflicts of interest.

Copyright © 2014 by Mutaz B. Habal, MD

ISSN: 1049-2275
DOI: 10.1097/SCS.0000000000000749

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

Downloaded from http://journals.lww.com/jcraniofacialsurgery by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIH04XMI0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 06/30/2024

TABLE 1. The Volume and Weight of the Rabbits' Left Hippocampuses

Hippocampus No. Rabbits	Weight of the Left Hippocampus, g	Volume of the Left Hippocampus, cm^3
1	0.27	0.26
2	0.26	0.25
3	0.29	0.28
4	0.31	0.31
5	0.27	0.27
6	0.30	0.29
7	0.28	0.28
Mean ± SD	0.28 ± 0.02	0.28 ± 0.02

(Giemsa azur eosin methylene blue solution—Merck) stain as described in the previous study of our study group.[8]

Stereological Analysis

All glass slides were fixed on a platform per specimen and the area of the hippocampal tissue on the slides was measured by 0.1 cm transparent point grid. All measurements were performed blinded to subject details and data of any other results.[10] The volume of the left half of the hippocampus was calculated by the formula below for each subject:

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

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

The coefficient of error (CE) of the study was calculated according to Nyengaard.[11]

RESULTS

Qualitative Findings

All studied brains and hippocampuses were in normal anatomical appearance. All parts and limits of the hippocampus tissue were clearly observed by Giemsa stain and calculated by stereological method (Fig. 1).

Quantitative Findings

The mean weight of the encephalon without cerebellum was 6.1 ± 0.1 g. The mean weight and the volume of the hippocampus were (mean ± SD) 0.28 ± 0.02 g and 0.28 ± 0.02 cm^3, respectively. There was no shrinkage of the tissues due to fixation effect. The mean coefficient of error for the stereological volume estimation of the hippocampus was 0.03 (Table 1).

DISCUSSION

The mechanism and the response against the clinical therapeutics in Alzheimer disease (AD) are investigated on the animal model. The rabbit model in AD is currently used. As a result of the AD, significant neuronal loss in frontal cortex, hippocampus, and cerebellum was observed in this rabbit model.[12] The hippocampus also plays an important role in various cognitive and affective events. Increasing problems show that these occasions morphologically spread along the dorsoventral and transverse axes of the hippocampus.[13] Investigations of functional differentiation of the hippocampus have displayed that the ventral part is related with emotional and anxiety-related functioning, and the dorsal part with memory processing. Thus, some researchers want to determine the whole hippocampal volume size to evaluate differences between the ventral and posterior hippocampus on some pathologies.[5,7] Hippocampal volume estimation using the Cavalieri method was previously achieved on the offspring of Guinea pigs and was found to be about 0.056 cm^3,[14] and on 2-month-old rats it was about 0.032 cm^3.[15] However, no detailed hippocampal volume estimation was detected in the literature. The finding in this study about the left hippocampal volume was 0.28 cm^3, and this result was obtained from the stereological estimation tool Cavalieri method. The estimates placed above were achieved by stereological methods. Meanwhile, stereological methods could be coupled with MR images. For example, in a study performed on human subjects, using the volume of the hippocampus estimated on the MR images, the volume of the left hippocampus was 2.5 cm^3.[16] From all these points, stereological methods are the best and perfect way against the other volumetric methods. One way to estimate the total volume of the tissue is by the Archimedean principle. This method can be run rapidly and simply, but it only allows the estimation of the total volume instead of the volume of the subcomponents and could not be combined with the MR images.[17] Another problem is the volume estimation of the small organ. Because of the small size of the organ, the surface tension becomes a challenge.[10,18] These are the side effects of the Archimedean principle. These problems could not find a position in the Cavalieri method. According to the literature, design-based stereology may be divided into evaluations of the global and local characteristics of tissues, the most significant of which are volume, number, connectivity, spatial distribution, and length of linear biological architecture.[6] The obtained CE level in this study was quite low. This low level could be obtained by slicing the tissue in frequent intervals and decreasing the distance between 2 points in the grid for counting a large amount of points per area.

If the volumes of the hippocampus are estimated, this contributes to the experimental researches, rabbit modeling mental disorder studies, and different rabbit researches. Another important point about the hippocampus of the rabbit is size. The rabbit hippocampus is quite big and, depending on its size, is easy to obtain individually. Thus, the estimates and researches on this tissue are relatively easy. Further studies may provide additional information on the relative value of the hippocampus volume compared to the hemisphere volume of the same animal. The study provides the first methodological substantiation of the experimental possibility to obtain volumetrically data using microscopic anatomic stereological technique. We believed that the findings and the applied method of the study may be useful for researchers.

REFERENCES

1. Greenstein B, Greenstein A. *Color Atlas of Neuroscience*. Stuttgart: Thieme, 2000:318
2. Scarabino T, Salvolini U. *Atlas of Morphology and Functional Anatomy of the Brain*. Berlin: Springer, 2006:6
3. Sandring S. *Gray's Anatomy.* 39th ed. Amsterdam: Elsevier, 2005: 405–410
4. Mu Y, Gage FH. Adult hippocampal neurogenesis and its role in Alzheimer's disease. A review. *Mol Neurodegeneration* 2011;22:85
5. Willard SL, Friedman DP, Henkel CK, et al. Anterior hippocampal volume is reduced in behaviorally depressed female cynomolgus macaques. *Psychoneuroendocrinology* 2009;34:1469–1475
6. 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
7. 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.

8. 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
9. Gocmen-Mas N, Pelin C, Canan S, et al. Stereological evaluation of volumetric asymmetry in healthy human cerebellum. *Surg Radiol Anat* 2009;31:177–181
10. Akosman MS, Ozdemir V. Capability of the Cavalieri method for volume estimation of the dog testis. *Eurasian J Vet Sci* 2010; 26:63–67
11. Nyengaard JR. Stereologic methods and their application in kidney research. *J Am Soc Nephrol* 1999;10:1100–1123
12. Woodruff-Pak DS, Agelan A, Del Vallec LA. Rabbit model of Alzheimer's disease: valid at neuropathological, cognitive, and therapeutic levels. *J Alzheimers Dis* 2007;11:371–383
13. Jinno S. Topographic differences in adult neurogenesis in the mouse hippocampus: a stereology-based study using endogenous markers. *Hippocampus* 2011;21:467–480.
14. Tveden-Nyborg P, Vogt L, Schjoldager JG, et al. Maternal vitamin C deficiency during pregnancy persistently impairs hippocampal neurogenesis in offspring of guinea pigs. *PLoS One* 2012;7: 484–488
15. Kaae SS, Chen F, Wegener G, et al. Quantitative hippocampal structural changes following electroconvulsive seizure treatment in a rat model of depression. *Synapse* 2012;66:667–676
16. Keller SS, Baker G, Downes JJ, et al. Quantitative MRI of the prefrontal cortex and executive function in patients with temporal lobe epilepsy. *Epilepsy Behav* 2009;15:186–195
17. Bas O, Acer N, Mas N, et al. Stereological evaluation of the volume and volume fraction of intracranial structures in magnetic resonance images of patients with Alzheimer's disease. *Ann Anat* 2009;191: 186–195
18. Gouletsou PG, Galatos AD, Leontides LS. Comparison between ultrasonographic and caliper measurements of testicular volume in the dog. *Anim Reprod Sci* 2008;108:1–12

Downloaded from http://journals.lww.com/jcraniofacialsurgery by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC1y0abggQZXdgGj2MwlZLeI= on 06/30/2024

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