Volume estimation of the rabbit thalamus.
Stereological microanatomical study

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The thalamus is the connection center of the brain. All synaptic impulses except olfactory are formed in the thalamus. Its current significance is its designation as the source of some diseases. Recent research shows that the thalamus has a critical role in schizophrenia[1] with changes in the volume and neuronal number in the thalamus in schizophrenia patients.[1] Total and individual volumetric estimation on the thalamus is also performed as much as investigations of the thalamic neuron number in schizophrenia.[1] Because of this finding, we aimed to identify a simple method for the rapid volume estimation on the rabbit thalamus in vitro on glass slides. The volume unit of the rabbit thalamus was measured in cubic centimeters. In vivo and in vitro volume of the brain compartments could be accurately, efficiently, cheaply, and simply estimated by the Cavalieri method in unbiased detail.

This study was carried out at the Afyon Kocatepe University, Afyonkarahisar, Turkey in 2010. The experimental design was the volume estimation of the left half of the thalamus on brains of 7 rabbits (1-1.5 years, 3-4 kg) by stereological volume measurement utilizing the Cavalieri principle. We obtained permission from the Local Ethics Committee for Animal Experiments of Afyon Kocatepe University. The animals were housed and all surgical operations performed in the Laboratory Animals Research Center of Afyon Kocatepe University. Other stainings and analyses were performed at the Stereology Laboratory of the Anatomy Department in the Veterinary Faculty of Afyon Kocatepe University. The animals were anesthetized with isoflurane and decapitated. The scalp skin of the rabbits was dissected 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 The soft tissue on the upper part of the calvary was retracted, and the skull dissected from the midline to where it reached the brain.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 were attentively dissected under a 4x loop microscope, and the specimens fixed in neutral buffered 10% formalin for 2 days. Each thalamus was measured before and after formalin fixation. The hemispheres were separated with a microtome blade from the midline after immersion fixation. The thalamus of the rabbit brain was easy to hold. After the total extirpation of the diencephalon, the thalamus was carefully dissected from the sulcus thalamo caudatus and sulcus transversus commissurae posterioris, and then separated from both the nucleus caudatus and the tectum mesencephali. The thalamic region was extracted gently to avoid tissue damage from the left hemispheres. The thalamus was sectioned in front of the joining point with the colliculus rostralis and colliculus caudalis, weighed and totally embedded in the paraffin. The thalamus in the paraffin block was serially sectioned at 40 µm thicknesses by rotary a microtome (Leica RM2155, Leica Inc., Nussloch, Germany) and every thirteenth slab chosen and stained by Giemsa (Giemsa's azur eosin methylene blue solution, Merck, Darmstadt, Germany.) stain. All glass slides were fixed on a platform and each specimen with the area of the whole thalamic tissue on the slides was measured by 0.1 cm transparent point grid. We did not perform cell discrimination and counting. All measurements were performed blinded to subject details and data of any other results. The volume of the left half of the thalamus was calculated by the following formula for each subject and descriptive statistical analysis was performed:

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

t is the section thickness (0.052cm), a/p is the area represented by a point in the grid (0.1cm x 0.1cm), ∑P is the total number of points hitting the surface area of sections. The statistically significant coefficient of error (CE) value of the study was under 0.05.

All studied brains and thalami were normal in anatomical appearance. All parts and limits of the thalamic tissue were clearly observed by Giemsa stain. The mean weight of the encephalon without cerebellum was 6.1±0.1gr. The mean weight of the thalamus was 0.24±0.02gr and mean volume was 0.23±0.02cm^3. There was no shrinkage of the tissues resulting from fixation effect. The mean coefficient of error for the stereological volume estimation of the thalamus was 0.03 (**Table 1**).

The MRI is especially used to estimate the total volume of the thalamus in research on mental illness and disorders such as schizophrenia and bipolar disorder.[2,3] The volumetric results obtained by MRI of the healthy human thalamus are nearly 7 cm^3.[1,3] The analysis of brain volume changes is complemented by the application of deformation-based morphometry, which revealed additional pathology in lesioned rats,

Disclosure. The authors declare no conflicting interests, and this study was not supported or funded by any drug company.

Table 1 - The weight and volume parameters per left thalamus in studied rats.

Thalamus no.	Weight of the left thalamus (gr)	Volume of the left thalamus (cm^3)
1	0.20	0.19
2	0.25	0.24
3	0.26	0.24
4	0.25	0.24
5	0.22	0.21
6	0.23	0.23
7	0.24	0.23
Mean±SD	0.24±0.02	0.23±0.02

particularly in the thalamus in neurological disorders.[1-3] To obtain accurate results, as in vitro experiments, the Cavalieri method should be used. This method successfully performed estimation of the volume of the thalamus in recombinant inbred mice.[4] The brains of the mice were sliced coronally, with the images taken from these serial slides containing the thalamic tissue. The areas of the tissues on these slides were estimated by the transparent test probe, and the value of 0.02 cm^3 was obtained for the total volume of the thalamus.[4] The only accurate, simple, and unbiased volume estimation is the Cavalieri method. The Cavalieri method prefers CE values less than 5%. In the present study, the obtained value was under this level demonstrating the quality of the sampling procedure. The obtained CE level was achieved by slicing the tissue at frequent intervals and decreasing the distance between 2 points in the grid to count a large amount of points per area.

In conclusion, this study demonstrates how to obtain quick data on the volume of the thalamus in rabbits. The research was performed on and limited to the left thalamus of 7 rabbits. The thalamus is an important part of the brain and may be affected by brain disorders.[5] Further studies will provide comprehensive information on the relative value of the thalamic volume compared to the hemisphere volume for the same animal when both sides are consistent. This paper provides a methodological substantiation of the experimental possibility to obtain thalamic volumetric data using a microscopical, anatomical, and stereological technique.

Received 6th March 2013. Accepted 23rd June 2013.

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