Neuroradiology (1999) 41: 493–496

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The assessment of postmortem brain volume; a comparison of stereological and planimetric methodologies

Received: 29 August 1997
Accepted: 15 December 1998

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Abstract We compared two methods of estimating the volume of 10 formalin-fixed brains using MRI. MRI was performed and total brain volume was then assessed using two distinct techniques: a stereological point-counting technique based on the Cavalieri principle, and an edge-tracing technique. The total brain volumes obtained using these two techniques were similar and correlated closely with each other ($r = 0.97$). Both methods could be optimised to a similar degree while maintaining the coefficient of error at an acceptably low level. However, the stereological assessment of brain volume required between 20 min and 30 min per brain, depending on the number of points per sampling grid, compared with 1 h per brain using the planimetric method. Thus, while planimetric and stereological approaches yield very similar results, the stereological method has the advantage of greater speed and, therefore, efficiency.

Key words Magnetic resonance imaging · Stereology · Planimetry

Introduction

Quantitation of the volume of biological specimens, especially complex biological organs such as the brain, is fraught with technical difficulties. Consequently, different approaches have been implemented to estimate various indices of brain size. The two main disciplines employed for measuring brain volumes include traditional morphometry and modern stereology.

Planimetry involves tracing around a region of interest on a computer screen. The actual volume of the tissue of interest is then calculated from the number of pixels encompassed within the traced regions in each of a series of images. In contrast, the stereological technique of point-counting, based on the cavalieri principle, estimates the volume of an object by dividing the object into a series of parallel sections of equal thickness and assessing the area of the object in a sample of the sections by point counting. Using either of these techniques, regions of interest can be sampled and their volume stimated in a simple and efficient manner [1, 2]. The statistical error associated with the methods [2] can be quantified and the experimental design, such as the number of grid points and/or slices counted, altered so that the efficiency of the procedure is maximised. The stereological point-counting technique has now been used successfully to assess postmortem brains [3], CT images [4] and also brain images obtained by MRI [5, 6]. Similarly, the planimetric method has been used successfully to assess hippocampal volumes [7, 8].

Our aim was to compare, with respect to intermethod reliability and efficiency, the planimetric and stereological techniques of postmortem brain volume estimation from MRI.

Materials and methods

We obtained 10 consecutive macroscopically normal brains from individuals with no known history of neurodegenerative disorder, epilepsy or alcohol abuse: four were male, mean age was 68 years, and age ranged from 44 to 82 years. Causes of death included myocardial infarction in six; rheumatic heart disease and septicaemia in one each; chronic obstructive airway disease in two. Brains were obtained at autopsy, and fixed in 10% formalin for 1 month.

Prior to MRI, each brain was removed from formalin and washed in running tap-water for several hours. Imaging was performed by one author (I.W.) using a clinical whole-body system operating at a field strength of 1.5 T. Each brain was placed in a standard circularly polarised head coil for radiofrequency transmission and reception with its convexity downwards. Images were acquired in the coronal (anatomical) plane using a dual spin-echo sequence (TR 1200 TE 20,90 ms; 4 acquisitions). An acquisition matrix of 256 × 256 over a 15-cm field of view with 0.5-cm-thick, contiguous slices gave in-plane resolution of 0.59 × 0.59 mm. Following data acquisition, all images were filmed on hard copy for the point-counting volume estimation and also transferred in digital form to an off-line work station for planimetric volume estimation. For the volume assessments we used proton-density images (TR 1200 TE 20 ms) which gave the clearest delineation of brain [9].

For each brain, total volume was determined separately by two independent assessors, one (DC) using stereology and one (KM) planimetry. For the purpose of this study, total brain volume, as assessed by both raters, related only to the mid- and forebrain. These were delineated from the hindbrain and cerebellum at the level of the caudal midbrain.

As previously described [10], using a hand-held mouse, the rater traced around the area of interest within a slice. Volume estimations were obtained from the images in digital format using an image display and analysis program written in-house [11]. The software calculated the number of pixels enclosed within the traced area and the process was repeated for each slice. Since the pixel dimensions and the slice thickness were known, the total brain volume can be estimated:

$$\text{total volume} = TPV \times SL \times (FOV^2/M)$$

where TPV is the total number of pixels covering the tissue of interest summed over all slices, SL the image slice thickness (0.5 cm), FOV the field of view (15 cm^2), and M the matrix size (256 × 256).

Total volumes for each brain were obtained on two occasions by tracing around the brain, excluding the cerebellum and brain stem. This procedure took approximately 1 hour per brain. These values formed the basis of the planimetric reliability measures.

Radiographic hard-copy proton-density-weighted images were placed on a X ray viewing box and, using a colour video camera, transferred to a video screen. A computer software package, served to superimpose a 20 × 20 point counting grid onto the captured image. For every slice the number of intersects with the brain was noted. Total brain volume was calculated from the Cavalieri formula:

$$\text{total volume} = P \times ((A \times SL)/NP)\,CF$$

where P is the sum of points overlapping the region of interest, A the total area of the grid (cm^2), SL the thickness of slice (0.5 cm), NP the number of points per grid (400 for the 20 × 20 grid and 100 for the 10 × 10 grid), and CF the constant correction factor required to normalise for magnification of the viewed hard-copy radiographic image (0.714). For this study, therefore, volume = P × (354 × 0.5/400) × 0.714 (cm^3).

Table 1 Total brain volume according to method of ascertainment

Case	MRI-based volume (cm^3)		
	stereology (20 × 20 grid)	stereology (10 × 10 grid)	planimetry
1	1013	999	967
2	1018	1069	1018
3	757	775	760
4	1120	1128	1096
5	983	1078	987
6	713	749	688
7	920	939	904
8	922	837	877
9	736	687	745
10	1087	1019	981
Mean (SD)	927 (145)	928 (155)	902 (133)

This procedure required approximately half an hour per brain. The total brain volume assessments were then repeated by one rater (DC) using a 10 × 10 point counting grid which took approximately 20 min to complete. These two assessments, using the different counting grids, formed the basis of stereological reliability measurements.

Finally, we calculated the coefficient of error of both planimetric and point-counting techniques according to whether all images or, every second, third or fourth image were assessed [1]. Statistical analyses were performed using SPSS for Windows (version 6.0).

Results

Mean values for total brain volumes calculated according to planimetric and stereological point-counting methods are listed in Table 1. Values for total brain volume, as obtained from planimetry and point counting of hard-copy images were highly correlated ($r = 0.97$, $P < 0.0001$). The value for the mean total brain volume obtained from the planimetric assessment, 902 cm^3 (SD 133 cm^3) was very similar to that obtained from point-counting of hard copy, 927 cm^3 (SD 145 cm^3).

The mean coefficient of error obtained by applying a 20 × 20 point-counting grid to all the hard-copy images was estimated at < 1%, that using a 10 × 10 point counting grid at 1.6%. However, estimation required less time (20 min per brain) using the 10 × 10 grid, than using the 20 × 20 point-counting grid (30 min). The planimetric assessment of images was associated with a coefficient of error of less than 1%. The values for the coefficient of error calculated for each method according to a sampling strategy such that each image or every second, third and fourth image were used to calculate the error are shown in Table 2.

There was good intrarater reliability for total brain volumes assessed by the planimetric method, as demonstrated by the close correlation ($r = 0.99$) between values obtained on two different occasions. Intrarater

Table 2 Coefficient of error derived according to sampling strategy and method of brain volume ascertainment

Number of images assessed	Coefficient of error		
	stereology (20×20 grid)	stereology (10×10 grid)	planimetry
All	0.011	0.013	> 0.01
Every 2nd	0.014	0.017	> 0.01
Every 3rd	0.017	0.021	> 0.01
Every 4th	0.021	0.024	> 0.01

reliability for total brain volumes obtained using the stereological technique was also good, as demonstrated by the close correlation between volumes obtained using the 10×10 and 20×20 point-counting grids ($r = 0.95$).

Discussion

We compared and correlated total brain volumes obtained from the same set of postmortem brains using planimetric and stereological approaches, and investigated the reliability and the efficiency of each technique. We found that the brain volumes obtained using these techniques are very similar, but that for a given sampling strategy, the stereological technique is faster and associated with an acceptably low coefficient of error. These quantitative findings were in contrast to our group's previous investigation which highlighted differences in qualitative MRI and neuropathological assessments in postmortem brains [12]. The further comparison of methods of assessment of postmortem brains were the impetus for the current investigation.

In a previous study, Mayhew and Olsen [6], measuring from MRI, showed that brain volume can be estimated efficiently using the Cavalieri principle and point-counting techniques such that the systematic sampling of just five or six slices of 0.6 cm thickness yielded an accurate volume estimate with a coefficient of error of less than 5%. Such systematic random sampling, combined with the use of the Cavalieri principle, has also been used to derive volume estimates from planimetric assessments of cerebral structures such as the hippocampus [7, 8] and is central to optimisation. The optimisation aims to reduce the time taken for the procedure, by reducing the number of slices assessed, following two rules. First, the number of images used can be reduced to every second, third, fourth or 'n'th section as long as the coefficient of error remains within an acceptable limit; and second, the first section to be assessed from the series of 'n' sections must be picked randomly, so that every section has an equal probability of being sampled and thus, the estimate is unbiased. This concept of unbiased estimations is central to stereology. While theoretically unbiasedness can also be influenced by the orientation of the plane of MRI sectioning, recent investigations using imaging to assess human organ volumes have found, unbiasedness was, in practice, independent of orientation [5, 13].

We found that the major difference distinguishing the planimetric and stereological methods is time. The point-counting technique was faster, requiring only half of the time needed for the equivalent planimetric assessment. Time saving is inherent in sampling rather than measuring the whole of an object. While both methods allow some degree of optimisation through slice sampling, in point-counting there is the additional opportunity to optimise through reducing the number of points per unit area. This allows flexibility in sampling and thus greater efficiency. Hence, in our study, this statistical error, the coefficient of error calculated from the point-counting, and planimetric assessments of *all* hard-copy-images, was less than 1% in each case. This low value indicated that we could optimise these methods. In the case of the point-counting technique, one way to do this was to reduce the intensity of the grid from 20×20 to 10×10. This yielded a similar volume in a shorter time, which correlated well with the brain volumes obtained from the planimetric assessment (95% for the 10×10 grid vs. 99.7% for 20×20 grid). The coefficient of error obtained using this 10×10 grid was still only 1.6%, which indicated that further optimisation was possible. Systematic random sampling of the point-counted and planimetrically assessed images was then undertaken to determine what amount of slice sampling was acceptable (e.g. by assessing every 'n'th image). For both methods, we found that even when assessing every fourth slice, we could obtain estimates of volume which would be statistically unbiased, and have an associated error of less than 2.5% (i.e. estimated volume would be within 2.5% of the actual volume). However, because the point-counting method was faster, and associated with a low error, it is more efficient. In the case of the stereological point-counting method, further optimisation can be obtained by altering the intensity of the point-counting grid. An additional methodological advantage of the point-counting-derived method is the statistically unbiased nature of the volume estimates yielded (i.e. the average error is zero).

Stereological estimate can be implemented alongside planimetric methods within a neuroimaging department as commercial software systems are becoming readily available. As a cheap, reliable alternative one could replace the stereological package with a simple set of transparent sheets upon which are copied grid points at different intensities. These could be placed over the images and viewed using a standard light box, thus allowing simple point counting. Furthermore, both planimetry and stereology, may be compared with the newly developing automated computer-thresholding techni-

ques, so as to confirm that algorithm-dependent procedures are reliable in boundary delineation. Indeed, boundary delineation is a problem even when applying planimetric and point-counting methods, as it boundary is inherently subjective. Unfortunately, in this investigation, the absence of a reference measurement of brain volume (such as by fluid displacement), makes it impossible to judge whether the planimetric or the point-counting method gives values closer to the true volume. In general, we found slightly larger brain volumes from the point-counting method, the most likely explanation for which is slight difference in boundary delineation by the two investigators.

The methodology involved in the Cavalieri estimation of volume is simple; all one requires is objects which may be imaged at uniform thickness. In clinical practice, this technique may be relevant to the serial assessment of neurodegenerative disorders, using MRI or CT, in which the subjective nature of decision-making regarding the presence of atrophy is notoriously difficult.

Acknowledgements D. Cotter is a MRC Clinical Training Fellow. We would like to thank Dr. Nigel Cairns of the MRC Brain Bank, Department of Neuropathology Institute of Psychiatry, for his help in providing tissue for this investigation.

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