ACTA STEREOL 1999; 18/2: 149-159
ORIGINAL SCIENTIFIC PAPER

COMPARISON OF COMPARTMENT VOLUMES ESTIMATED FROM MR IMAGES AND PHYSICAL SECTIONS OF FORMALIN FIXED CEREBRAL HEMISPHERES

Clare E. MACKAY[1], Bente PAKKENBERG[2], Neil ROBERTS[1]

[1]Magnetic Resonance and Image Analysis Research Centre (MARIARC), University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, UK and
[2]Neurological Research Laboratory, Bartholin Instituttet, Kommunehospitalet, 1399 Copenhagen K, Denmark

ABSTRACT

Magnetic Resonance Imaging (MRI) offers considerable promise for the quantitative study of the brain. We have investigated the application of MRI to measure the volume of six formalin-fixed cerebral hemispheres and their internal compartments. In particular, we have compared estimates of the volume of i) cortex (COR), ii) sub-cortex (SUBCOR, i.e. white matter plus central grey matter) and iii) whole cerebral hemisphere (i.e. TOTAL) obtained from MR images with those obtained from physical sections of the same specimens. The method used for volume estimation was the Cavalieri method of modern design-based stereology, which is mathematically unbiased. Volume estimates were obtained from the physical sections by one observer and from the MR images by another observer. Application of paired t-tests revealed no significant differences between the mean volume of COR, SUBCOR and TOTAL obtained from the physical sections and MR images ($p > 0.05$). A tendency was, however, observed for estimates of SUBCOR and TOTAL obtained from the MR images to be lower than those obtained from the physical sections. In two specimens the under-estimation is significant in that the difference in the volumes of SUBCOR estimated from the physical sections and MR images is much greater than the predicted standard error on the respective volume estimates. We recommend that further investigations be performed to evaluate and compare the volume of the cerebral hemispheres and their internal compartments estimated from both physical sections and MR images of formalin-fixed specimens.

Key words: brain, Cavalieri method, grey matter, Magnetic Resonance Imaging (MRI), point counting, stereology, white matter.

INTRODUCTION

This study was conceived as a comparison of the volume of (i) cortical grey matter (neocortex plus archicortex), (ii) white matter and (iii) central grey matter nuclei (e.g. thalamus, putamen, etc.) estimated from MR images of formalin fixed cerebral hemisphere specimens with volumes estimated from physical sections of the same specimens. However, it quickly became apparent, that while providing a reasonable depiction of the boundary between cortex

and white matter in formalin-fixed specimens, proprietary 3-D protocols available on a 1.5 Tesla whole body MR imaging system do not provide a sufficiently clear depiction of the central grey nuclei to make estimation of their total volume from these images a viable prospect. We have therefore compared estimates of the volume of compartment (i) above, which we call cortex (COR), the union of compartments (ii) and (iii) above, which we call sub-cortex (SUBCOR), and their combination, which we call TOTAL, estimated from MR images with corresponding volumes estimated from physical sections for six formalin-fixed specimens.

A variety of approaches have been used for measuring brain structures on MR images obtained in-vivo. Commonly, computer-based planimetric techniques have been employed, to measure, for example, hippocampal volume in clinical investigation of patients with temporal lobe epilespy (e.g. Cook et al, 1992), schizophrenia (e.g. Shenton et al, 1992) and Alzheimer's disease (e.g. Killiany et al, 1993). Planimetric techniques are too tedious to employ for outlining boundaries of the gyri and sulci on MR images. However, the Cavalieri method of modern design-based stereology (CruzOrive, 1985; Gundersen and Jensen, 1987; Rosen and Parry, 1990; Mayhew and Olsen, 1991; Roberts et al, 1993; Roberts et al, 1994; Cruz-Orive, 1997; Cruz-Orive, 1999; Gundersen et al, 1999) has been used in combination with point counting to estimate the volume of the cerebellum (Escalona et al, 1990), the frontal lobes (Sheline et al, 1996) and whole cerebral hemisphere (Regeur and Pakkenberg, 1989; Mackay et al, 1998).

In order that it can be described as clinical useful, an MR based volume estimation procedure should be both precise and based on unbiased principles, and this has been demonstrated for organs and compartments of the living human body in several studies employing the Cavalieri method in this laboratory (Roberts et al, 1993; Roberts et al, 1994; Light et al, 1995; Gong et al, 1998). The present study builds on the work of Mayhew and Olsen (1991) who, using MRI and the Cavalieri method, estimated the volume of a single formalin-fixed cerebral hemisphere to be 3.3% less than the volume obtained by fluid displacement. We have studied six forman-fixed cerebral hemisphere specimens. Our objective was to investigate whether there were any systematic differences between volume estimates obtained from MR images and physical sections of these specimens.

ESTIMATION OF VOLUME BY THE CAVALIERI METHOD

By using the Cavalieri method an unbiased estimate of the volume of a structure of arbitrary shape and size may be obtained efficiently and with know precision. The method requires that, beginning from a uniform random starting position within the sectioning interval, the structure is sectioned from end to end with a series of parallel planes a constant distance apart, whereupon an unbiased estimate of volume is obtained by multiplying the total area of the profiles through the structure on all the sections by the sectioning interval, i.e.

$$\text{est}_1 V = T \,.\, (A_1 + A_2 + \ldots\ldots + A_n), \text{ cm}^3 \qquad (1)$$

where A_1, A_2, ... A_n denote the section areas in cm^2 and T is the sectioning interval in cm for the n consecutive sections. Several studies have shown that interactive point counting

techniques represent a more reliable and efficient approach than planimetric drawing techniques for obtaining the required section areas (Mathieu et al, 1981; Gundersen et al, 1981). The point counting method consists of overlying each section with a regular grid of test points, which is randomly positioned to avoid bias (see Figure 1). After each superimposition, the number of test points hitting the structure of interest on the sections is counted, and the unbiased estimator becomes

$$est_2V = T \cdot a/p \cdot (P_1+P_2+\ldots\ldots+P_n),\ cm^3 \qquad (2)$$

where P_1, P_2, ... P_n denote the point counts and *(a/p)* represents the area associated with each test point, corrected for any change of scale of the image as it is displayed on the computer screen. The area of each section, A_i, is now estimated by *(a/p)* x P_i. The subscript 2 in est_2V indicates that the volume is estimated by a two stage process, namely sectioning and point counting.

The precision of a volume estimate obtained using the Cavalieri sections method may be measured by its Coefficient of Error (CE) or 'relative standard error'. However, prediction of the CE for systematic sections is not straightforward. Since consecutive section areas are not independent quantities, conventional statistical formulae can not be applied to determine the variance of their sum. A reasonable (but not mathematically unbiased) error prediction formula for the Cavalieri sections method was developed by Gundersen and Jensen (1987) based on the theoretical work of Matheron (1965, 1971). A plot of the area of the structure of interest on consecutive systematic sections is termed the measurement function. The variance of an estimate obtained by systematic sampling corresponds to the difference between the integral of the covariogram of the true function and the discrete approximation of this integral derived from the section area measurements (Yates, 1948; Moran, 1950, and see pages 318 to 319 in Cruz-Orive, 1989). Since the true function is not known it has to be modelled. Critically, Matheron (1965; 1971) showed that the relevant difference could be evaluated from the section area measurements by using so-called Euler-MacLaurin formula to model the behaviour of the covariogram near the origin. Gundersen and Jensen (1987) used this approach to develop a method for predicting the precision of volume estimates obtained using the Cavalieri method and demonstrated that systematic sampling is more efficient than random sampling by a factor that is generally equal to the square root of the number of sections analyzed. Originally it was supposed that the contribution of point counting to a Cavalieri estimate of volume could be ignored relative to that due to sectioning (Gundersen and Jensen, 1987; Pakkenberg et al, 1989; Pache et al, 1993).

Souchet (1995), Kieu (1997), Kieu et al (1999), and Gundersen et al (1999) have developed this formula for 3D smooth objects. The theory, which is based on Matheron´s theory of "regionalized variables" (1965, 1971), reveals that the variance depends on the smoothness properties of the measurement function:

$$CE = \frac{\left[\frac{1}{240} * 3\left(\sum P_i^2 - (0.0724 * (b - a) * \sqrt{n} * \sum P)\right) - 4\sum P_iP_{i+1} + \sum P_iP_{i+2}\right] + (0.0724 * (b - a) * \sqrt{n} * \sum P)}{(\sum P)^2} \qquad (3)$$

This formula can be rewritten as (see, Cruz-Orive, 1999):

$$CE^2(\hat{V}) = \left(\tfrac{1}{240}\right) * \left(3 * \sum_{i=1}^{n} P_i^2 + \sum_{i=1}^{n-2} P_i * P_{i+2} - 4 * \sum_{i=1}^{n-1} P_i * P_{i+1}\right) \cdot P_i^2$$
$$+\left(1 - \tfrac{3}{240}\right) * 0.0724 * \overline{B}\sqrt{\overline{A}} * \left(\sum_{i=1}^{n} P_i\right)^{-2} \qquad (4)$$

which has been used in this study.

Calculation of the CE using Eqs. (3) and (4) requires knowledge of the value of a dimensionless shape coefficient $B\sqrt{A}$, which is equivalent to the mean boundary length of the profiles divided by the square root of their mean area (Matern (1985)), and is a measure of the average shape of the profiles through the structure of interest on the sections.

MATERIAL AND METHODS

Six cerebral hemisphere specimens bequeathed by subjects with no history of dementia, alcoholism or other neurodegenerative disorder (3 males, 3 females; mean age = 70 years) were fixed in formalin and set in separate blocks of agarose gel. The specimens were imaged with a 1.5 Tesla SIGNA (General Electric, Milwaukee, U.S.A.) whole body MR imaging system using a proprietary quadrature head coil. One hundred and twenty four coronal T1-weighted images were obtained using a 3D Spoiled Gradient Echo (SPGR) pulse sequence (TR = 34 ms, TE = 9 ms, flip angle = 450, NEX = 2, acquisition time = 27 mins and 52 secs). The FOV of the images is 16 cm. Each image refers to a 1.6 mm thick slice of tissue and consecutive slices are contiguous. Images were transferred to a SPARC 10 workstation (SUN Microsystems, CA, USA) and input to ANALYZE (MAYO Foundation, Minnesota, USA) software. The 256 x 256 x 124 acquired voxels of side 0.625 mm x 0.625 mm x 1.6 mm were linearly interpolated to 256 x 256 x 317 cubic voxels of side 0.625 mm. Contrast on conventional MR images results from differences in T1 and T2 relaxation times and the abundance (i.e. proton density) of mobile hydrogen nuclei in tissues. In-vitro, white matter has a longer T1 than grey matter and so appears the darker of the two tissues on the T1-weighted images.

For each specimen the volumes of the compartments COR and SUBCOR were estimated by point counting on systematic random series of MR images. The combined volume of COR and SUBCOR is equal to the volume of the whole cerebral hemisphere, which in our study is the volume of the whole specimen (i.e. TOTAL). The compartment SUBCOR, and therefore also TOTAL, excludes the volume of the cerebral ventricles. Beginning with a random start, point counting was performed at section intervals of 4.375 mm (i.e. on every seventh image in the reformatted datasets), using a square grid of size 15 pixels, which is equivalent to 9.375 mm. Values of the dimensionless shape coefficient ($B\sqrt{A}$) estimated for COR, SUBCOR and TOTAL from the MR images of specimen K023 were 19.3, 11.5 and 7.7, respectively. The volumes COR and SUBCOR were estimated a further four times from the

volume of specimen K023. In this example the sectioning interval is a factor of 2.6 greater than was employed in the study to be reported below. The six systematic random coronal sections in Figure 1 are separated by 45 tissue slices, each 0.625 mm thick, which corresponds to a distance, T, of 2.8125 cm. The grey-scale images are overlain with a test system with a separation between test points of 15 pixels, which corresponds to a distance of 0.9375 cm. The area associated with each grid point, a/p, is thus 0.8789 cm^2. The number of points recorded as lying in brain tissue on consecutive sections is 17, 35, 49, 44, 29 and 12, which gives a total of 186. An unbiased estimate of the TOTAL volume of the specimen is therefore $186 \cdot 0.8789 \cdot 2.8125 = 459.8\ cm^3$.

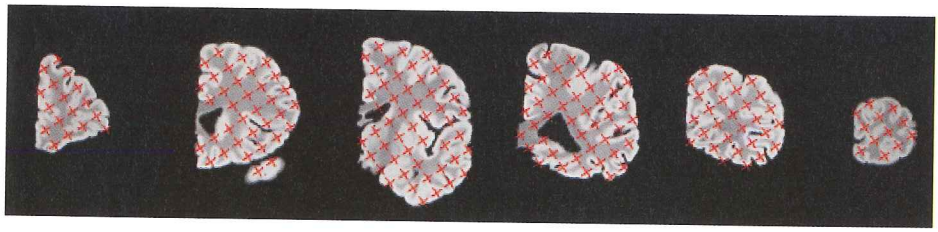

Fig. 1. Illustration of the application of the Cavalieri method to estimate the volume of cerebral hemisphere specimen K023. An unbiased estimate of 459.8 cm^3 is obtained from the total of 186 point counts on six systematic random sections.

After MR imaging the six formalin-fixed cerebral hemispheres were returned to the laboratory in Denmark and physically sectioned using a high precision cutting tool. Horizontal lines drawn in marker pen on the block of gel ensured that physical sectioning was carried out in approximately the same direction as the MR imaging, pilot studies in this laboratory having shown that cerebral volume estimates are more efficiently obtained by sectioning in the coronal than the axial or sagittal orientation. Each of the contiguous physical slices is 4.54 mm thick. The cut sections were overlain with a transparent film containing a test system for point counting (grid spacing = 1.125 cm^2 for COR and 4.5 cm^2 for SUBCOR). Point counting was performed by a different observer than had analyzed the MR images. However, one of the specimens (K023) was returned to Liverpool and estimates of the volume of COR, SUBCOR and TOTAL were obtained from the physical sections by the first observer (grid spacing = 10.1 mm).

Paired t-tests were performed to test for significant differences between volume estimates obtained for the six specimens by different observers on the MR images and physical sections.

RESULTS

The volumes obtained for COR, SUBCOR and TOTAL from the MR images (CM1) and

physical sections (LS) are presented in Table 1.

Application of paired t-tests revealed no significant differences between the volumes of COR, SUBCOR and TOTAL obtained from the physical sections and MR images ($p > 0.05$). However, whereas the mean of the volume estimates of COR obtained from the MR images is within 1% of the value obtained from the physical sections, for SUBCOR the mean volume obtained from the MR images is 12% lower than that obtained from the physical sections, so that the estimated volume of TOTAL is also lower on the MR images than physical sections (i.e. average of 6%) (see Figure 2). It is possible that the tendency for under-estimation has arisen from two outliers (i.e. specimens K096 and K245) in which the difference between the volume of SUBCOR estimated from the MR images and physical sections is significant.

For each specimen estimates of TOTAL volume were obtained from the MR images on a further four occasions (i.e. CM2 to CM5 in Table 1). In the first trial the section interval was 4.375 mm and in the other four trials the section interval was 9.375 mm. Not unexpected, the estimates are highly consistent (i.e. empirical CE of 1.7%). The five estimates of COR and SUBCOR obtained from the MR images of specimen K023 using a section interval of 9.375 mm are also highly consistent (i.e. CE's of 4.0% and 2.2%, respectively).

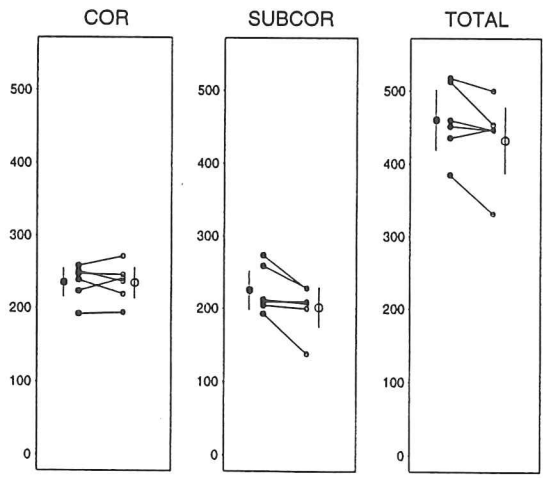

Fig. 2. The volume estimates obtained from the physical sections are denoted by filled circles, and the mean volume of the compartment is shown to the left of the six individual estimates. The volume estimates obtained from the MR images are denoted by open circles, and the mean volume of the compartment is shown to the right of the six individual estimates.

Table 1. Cavalieri estimates of the volume of COR, SUBCOR and TOTAL obtained from physical sections (LS and CM) and MR images (CM1 to CM5) of six formalin-fixed cerebral hemisphere specimens. The percentage CE´s predicted using Eqs. (3) and (4) are given in parenthesis. The mean values for each compartment are also presented together with the percentage Coefficient of Variation; i.e. standard deviation divided by the mean.

		Physical Sections		Magnetic Resonance Imaging				
		LS	**CM**	**CM1**	**CM2**	**CM3**	**CM4**	**CM5**
K023	**COR**	223.5	234.4	241.5	230.8	222.6	239.1	244.0
	SUBCOR	212.3	209.6	206.5	202.0	202.8	193.7	196.2
		(7.4)	(1.9)	(2.0)	(3.1)	(3.0)	(3.2)	(3.2)
	TOTAL	435.8	444.0	448.0	432.8	425.4	432.8	440.2
		(3.9)	(0.8)	(0.9)	(1.4)	(1.4)	(1.4)	(1.4)
K032	**COR**	251.0		236.9				
		(2.8)		(2.4)				
	SUBCOR	208.9		209.6				
		(7.8)		(2.0)				
	TOTAL	459.9		446.5	444.1	445.8	448.2	447.4
		(3.9)		(0.9)	(1.4)	(1.4)	(1.4)	(1.4)
K096	COR	192.0		193.8				
		(3.3)		(2.7)				
	SUBCOR	192.9		138.0				
		(7.5)		(2.7)				
	TOTAL	384.9		331.8	349.4	341.1	313.9	337.8
		(4.1)		(1.1)	(1.6)	(1.7)	(1.7)	(1.7)
K245	**COR**	239.0		226.5				
		(2.7)		(2.5)				
	SUBCOR	273.8		227.6				
		(6.3)		(1.9)				
	TOTAL	512.8		454.1	445.8	463.9	455.7	452.4
		(3.5)		(1.0)	(1.4)	(1.3)	(1.4)	(1.3)
K575	**COR**	258.4		271.8				
		(3.0)		(2.2)				
	SUBCOR	259.2		228.4				
		(6.9)		(1.9)				
	TOTAL	517.6		500.2	497.7	502.6	496.9	499.3
		(3.2)		(0.9)	(1.3)	(1.3)	(1.3)	(1.3)
K648	**COR**	247.3		246.1				
		(3.4)		(2.3)				
	SUBCOR	204.3		199.9				
		(7.5)		(2.1)				
	TOTAL	451.6		446.0	461.4	452.4	436.7	459.8
		(3.4)		(0.9)	(1.3)	(1.3)	(1.4)	(1.3)
mean	**COR**	235.2		236.1				
		(10.2)		(10.8)				
	SUBCOR	225.1		201.7				
		(14.7)		(16.5)				
	TOTAL	460.4		437.8	438.5	438.5	430.7	439.5
		(10.8)		(12.8)	(11.2)	(12.3)	(14.3)	(12.3)

DISCUSSION

Our observation that paired t-tests found no significant difference between the mean volumes of COR, SUBCOR and TOTAL estimated from the MR images and physical sections provides support for the application of MR imaging in quantitative studies of compartment volumes in studies of formalin-fixed post-mortem specimens. There was, however, a tendency for SUBCOR to be under-estimated on the MR images compared to the physical sections that merits further investigation. Regular quality control checks and previous validation studies in this laboratory (Light et al, 1995) make it unlikely that this has arisen from miss-calibration of the MR system. The finite thickness of the slice of tissue imaged can represent a limitation in studies using MR imaging. If the signals arising from different tissue compartments can not be separated within each voxel, then an artefact known as partial voluming is produced. This uncertainty in the exact contents of any voxel is an inherent property of the discretised image and would even exist if the contrast between tissues were infinite. Partial voluming of high signal intensity grey matter with lower signal intensity white matter may have tended to move the boundary between COR and SUBCOR inwards and be responsible for the tendency for under-estimation of SUBCOR on the MR images. An alternative explanation could be that MRI depicts a different physico-chemical boundary between COR and SUBCOR than the one apparent on the physical sections. However, neither of these suggestions can explain why the mean TOTAL volume of the specimens estimated from the MR images tended to be lower than that obtained from the physical sections. Partial voluming artefacts are minimised in this study via the use of high resolution (i.e. 3D) imaging protocols for which the slice thickness is 1.6 mm. We recommend that further studies are performed to establish the accuracy with which the volume of the cerebral hemispheres and their internal compartments can in practice be obtained from both physical sections and MR images of formalin-fixed specimens.

The volumes obtained for COR, SUBCOR and TOTAL in the six specimens investigated in the present study are not the volumes that would have been present in-vivo. The effect of formalin-fixation on brain tissue volume has been discussed by Mouritzen Dam (1979). From a study of formalin fixed specimens Pakkenberg and Gundersen (1997) provide a formula by which the number of neurons in the neocortex can be predicted from knowledge of neocortical volume. This formula could be used in combination with MRI to provide estimates of the number of neurons in the neocortex of in-vitro specimens. No shrinkage was found between the overall volume of the fresh brains and the volume of the 94 fixated brains at the time they were processed (unpublished data). A possible differential shrinkage, not affecting the total volume, can, however, never be excluded. Other problems include low contrast of the fixated tissue. The lower the contrast (cortex/white matter) the lower the precision of the estimate.

To our knowledge, the present study is the first to investigate the volumes of COR and SUBCOR on MR images of formalin-fixed cerebral hemisphere specimens. From the data corresponding to LS and CM1 in Table 1, we obtain average values over all six specimens of 1.05 (standard deviation, SD, = 12%) and 1.19 (SD = 11%) for the ratio of COR to SUBCOR on physical sections and MR images, respectively. Sisodiya et al (1996) have measured the volume of COR and SUBCOR on MR images obtained in-vivo for 33 healthy subjects of median age 29. The ratio of COR to SUBCOR was on average lower in 11 females (1.02, SD = 29%) than in 22 males (1.06, SD = 26%), and lower in the right than the left cerebral hemisphere in both sexes.

Computer based image analysis techniques are being developed for the measurement of tissue compartment volumes from MR images obtained in-vivo. There are two main

approaches. The first uses a novel data acquisition strategy to attempt to determine the relative amounts of grey matter, white matter and CSF on a pixel by pixel basis in multi-slice (i.e. 2D) series of MR images (e.g. Rusinek and Chandra, 1993), whereas the latter use statistical techniques to determine 3D grey and white matter probablity maps from high resolution (i.e. 3D) MR data sets (e.g. Ashburner and Friston, 1997). These methods could be developed for application in in-vitro studies.

Morphometric studies of the living brain using MRI may encounter effects not observed in this study of formalin-fixed specimens. In-vivo the T1 of white matter is shorter than that of grey matter and the relative signal intensities of the compartments on T1-weighted images is the reversal of that found in fixed specimens. In addition, Wiener et al (1996) have reported 5% changes in MR image signal intensity with a period of 4 seconds, and random, respiratory and cardiac synchronised brain motion, and CSF pulsations, although small, occur throughout any MR acquisition obtained for a living subject (Feinberg, 1987; Enzmann and Pelc, 1992; Poncelot, 1993). These inherent movements represent limits to the accuracy with which the volume of brain tissue compartments can in practice be estimated in-vivo.

ACKNOWLEDGEMENT

We sincerely thank the Hartmann Brothers Foundation, the Foundation of Director E. Danielsen and Wife, and the Copenhagen Hospital Corporation Research Council for financial support.

REFERENCES

Ashburner J, Friston K. A Bayesian model for robust tissue classification. Proceedings of the Third Human Brain Mapping Conference, Copenhagen, 1997.

Cook MJ, Fish DR, Shorvon SD, Straughan K, Stevens JM. Hippocampal volumetric and morphometric studies in frontal and temporal lobe epilepsy. Brain 1992; 115: 1001-15.

Cruz-Orive LM. Estimating volumes from systematic hyperplane sections. J Appl Probab 1985; 22: 518-30.

Cruz-Orive LM. On the precision of systematic sampling: a review of Matheron's transitive methods. J Microsc 1989; 160: 89-95.

Cruz-Orive LM. Systematic sampling in stereology. Bull Int Stat Inst 1993; 55: 451-68.

Cruz-Orive LM. Stereology of single objects. J Microsc 1997; 186: 93-107.

Cruz-Orive LM. Precision of Cavalieri sections and slices with local errors. J Microscopy 1999; 193: 182-98.

Enzmann DR, Pelc NJ. Brain motion: measurement with phase contrast MR imaging. Radiology 1992; 185: 653-60.

Escalona PR, McDonald WM, Doraiswamy PM, Boyko OB, Husain MM, Figiel GS, Laskowitz D, Ellinwood EH, Krishnan KRR. In-vivo stereological assessment of human cerebellar volume: Effects of Gender and Age. Am J Neuroradiol 1990; 12: 927-29.

Feinberg DA. Human brain motion and CSF circulation demonstrated with MR velocity mapping. Radiology 1987; 163: 793-99.

Gong QY, Roberts N, Garden AS, Whitehouse GH. Fetal and fetal brain volume estimation in the third trimester of human pregnancy using gradient echo MR imaging. Magn Reson Imaging 1998; 16: 235-40.

Gundersen HJG, Jensen EB. The effficiency of systematic sampling in stereology and its prediction. J Microsc 1987; 147: 229-63.

Gundersen HJG, Boysen M, Reithensen A. Comparison of semi-automatic digitizer tablet and

simple point counting performance in morphometry. Virchows Arch [Cell Pathol] 1981; 37: 317-25.

Gundersen HJG, Jensen EBV, Kieu K, Nielsen J. The efficiency of systematic sampling in stereology – reconsidered. J Microsc 1999; 193: 199-211.

Kieu K. Three lectures on systematic geometric sampling. Department of Theoretical Statistics, University of Aarhus, Denmark, Memoirs 13/1997.

Kieu K, Souchet S, Istas J. Precision of systematic sampling and transitive methods. J Stat Planning and Inference 1999; 77: 263-79.

Killiany RJ, Moss MB, Albert MS, Sandor T, Tieman J, Jolesz F. Temporal lobe regions on magnetic resonance imaging identify patients with early Alzheimer disease. Arch Neurol 1993; 50: 949-54.

Light K, Roberts N, Whitehouse GH, Edwards RHT. Unbiased and efficient estimation of bladder volume with MR imaging. Magn Reson Imaging 1995; 5: 33-41.

Mackay CE, Roberts N, Mayes AR, Downes JJ, Foster JK, Mann D. An exploratory study of the relationship between face recognition memory and the volume of medial temporal lobe structures in healthy young males. Behav Neurol 1998; 11: 3-21.

Matheron G. Les Variables Regionalisees et Leur Estimation, Masson et Cie., Paris, 1965.

Matheron G. The Theory of Regionalised Variables and its Applications, Les Cahiers du Centre de Morphologie Mathematique de Fontainebleau, No 5. Fontainebleau: Ecole National Superieure des Mines de Paris, 1971.

Mathieu 0, Cruz-Orive LM, Hoppeler H, Weibel ER. Measuring error and sampling variation in stereology: comparison of the efficiency of various methods of planar image analysis. J Miscrosc 1981; 121: 75-88.

Mayhew TM, Olsen D. Magnetic resonance imaging and model free estimates of brain volume. J Anat 1991; 178; 133-44.

Moran PAP. Numerical integration by systematic sampling. Proceed Cambridge Philosoph Soc 1950; 46; 111-15.

Mouritzen Dam A. Shrinkage of the brain during histological procedures with fixation in formaldehyde solutions of different concentrations. J Hirnforsch 1979; 20: 115-19.

Pache JC, Roberts N, Zimmerman A, Vock P, Cruz-Orive LM. Vertical LM sectioning and parallel CT scanning designs for stereology: Application to human lung. J Microsc 1993: 170: 3-24.

Pakkenberg B, Gundersen HJG. Neocortical neuron number in humans: Effect of sex and age. J Comp Neurol 1997: 384: 312-20.

Pakkenberg B, Boesen J, Albeck M, Gjerris F. Unbiased and Efficient Estimation of total ventricular volume of the brain obtained from CT Scans by a stereological method. Neuroradiology 1989: 31: 413-17.

Poncelot BP. (192) Brain parenchyma measured with EPMRI. Radiology 1992; 185: 645-51.

Regeur L, Pakkenberg B. Optimizing sampling designs for volume measurements of components of human brain using a stereological method. J Microsc 1989; 155: 113-21.

Roberts N, Cruz-Orive LM, Reid NMK, Brodie DA, Bourne M, Edwards RHT. Unbiased estimation of human body composition by the Cavilieri method using magnetic resonance imaging. J Microsc 1993; 171: 239-53.

Roberts N, Garden AS, Cruz-Orive LM, Whitehouse GH, Edwards RHT. Estimation of fetal volume by magnetic resonance imaging and stereology. Brit J Radiol 1994; 67: 1067-77.

Rosen GD, Parry JD. Brain volume estimation from serial section measurements: a comparison of methodologies. J Neurosc Meth 1990; 35: 115-24.

Rusinek H, Chandra R. Brain tissue volume measurement from magnetic resonance imaging. Invest Radiol 1993; 28: 890-95.

Sheline YI, Black KJ, Lin DY, Christensen GE, Gado MH, Brunsden BS, Vannier MW.

Stereological MRI volumetry of the frontal lobe. Psychiat Res: Neuroimag Sect 1996; 67: 203-14.

Shenton ME, Kikinis R, Jolesz FA, Pollak SD, LeMay M, Wible CG, Hokama H, Martin J, Metcalf D, Coleman M, McCarley RW. Abnormalities of the left temporal lobe and thought disorder in schizophrenia. New Engl J Med 1992; 327: 604-12.

Sisodiya S, Free S, Fish D, Shorvon S. MRI-based surface area estimates in the normal adult human brain: evidence for structural organisation. J Anat 1996; 188: 425-38.

Small CS, Peterson DI. The reliability of dimensions of formalin-fixed brains. Neurology 1982; 32: 413-15.

Souchet S. Precision de l'estimateur de Cavalieri. Rapport de stage, D.E.A. de Statistiques et Modeles Aleatoires a la France, Universite Paris-VII. Laboratoire de Biometrie, INRA-Versailles, 1995.

Wiener E, Schad LR, Baudendistel T, Essig M, Muller E, Lorenz WJ. Functional MR imaging of visual and motor cortex stimulation at high temporal resolution using a flash technique on a standard 1.5 Tesla scanner. Magn Res Imaging 1996; 14: 477-83.

Yates F. Systematic sampling. Philosoph Transact Royal Soc (London) A, 1948; 241: 345-77.