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Magnetic resonance imaging (MRI) and model-free estimates of brain volume determined using the Cavalieri principle

TERRY M. MAYHEW* AND DAG RUNE OLSEN

Department of Human Morphology, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK, and Department of Medical Physics and Image Analysis Laboratory, The Norwegian Radium Hospital, N-0310 Oslo 3, Norway

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INTRODUCTION

Magnetic resonance imaging techniques make it possible to abstract quantifiable information from slices taken through different parts of the living body. This 'imager slicing' approach is directly comparable to the 'physical or mechanical slicing' which is routine in anatomy and pathology both at the macroscopical and microscopical levels. Just as with physical slices, structures appearing within MRI slices may be quantified to abstract useful 3-dimensional information.

Earlier attempts to quantify organ volumes from MRI slices and by computed tomography (CT) have tended to rely on simple models to approximate organ shape and to test the 'accuracy' of the measurement methods (Kvist, Sjöström & Tylen, 1986; Ashtari *et al.* 1990; Fowler *et al.* 1990*a*, *b*). This approach is not unbiased in general and alternative, design-based, methods are preferable because they are independent of structure shape and spatial orientation (see Mayhew, 1989, 1990). In fact, the necessary unbiased estimators are all available already in the stereological literature (Weibel, 1979; Mayhew, 1983, 1991; Gundersen *et al.* 1988*a*, *b*). Unbiased estimation is guaranteed by generating slices in the proper ways.

A convenient, efficient and unbiased way of slicing arbitrary objects for volume estimation is the Cavalieri principle (Gundersen & Jensen, 1987). This has been employed recently on physical slices of brains (Pakkenberg & Gundersen, 1988; Henery & Mayhew, 1989; Regeur & Pakkenberg, 1989; Mayhew, Mwamengele & Dantzer, 1990) and other organs (e.g. Michel & Cruz-Orive, 1988). An example of its application to estimate ventricular volume on CT slice images of hydrocephalic brains has been reported (Pakkenberg, Boesen, Albeck & Gjerris, 1989). Since the Cavalieri method depends on estimating the areas of properly randomised slices, apparently similar methods which do not specify the random sampling protocol (e.g. Ashtari *et al.* 1990) cannot be considered unbiased in general.

The purpose of this study was threefold: (1) to assess the possibility of generating Cavalieri estimates of brain volume from MRI slices: (2) to assess the random errors attributable to alternative ways of sampling such slices; and (3) to relate those errors to the observed variation in volume between brains.

* Correspondence to Professor T. M. Mayhew, Department of Human Morphology, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK.

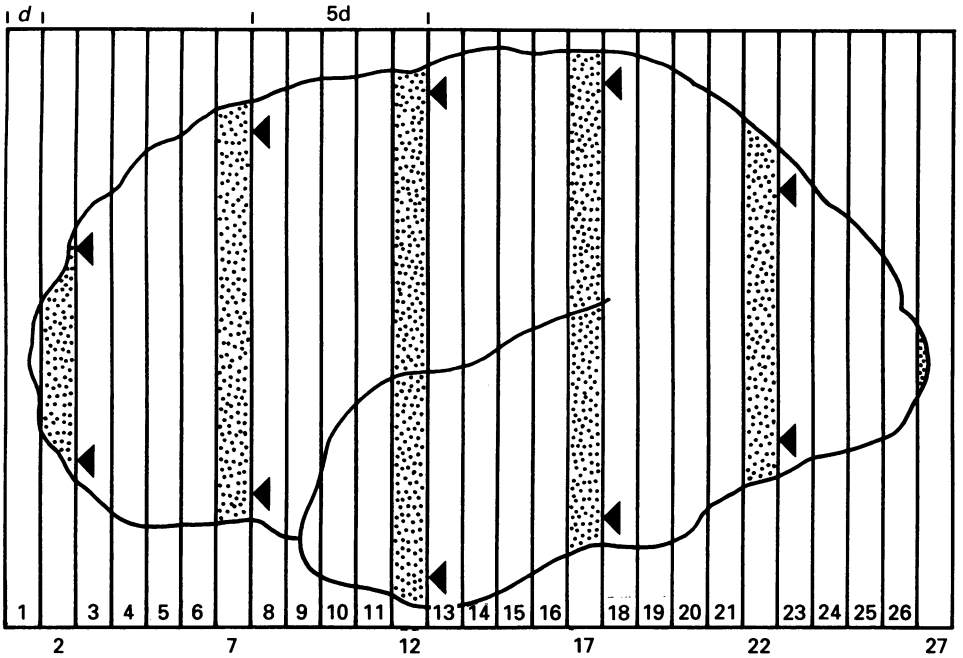

Fig. 1. Cavalieri estimation of volume. A brain (frontal pole to the left, occipital pole to the right) is divided into uniform random slices (1–27) of thickness *d*. A systematic sample of these slices (1 in 5, shown stippled) is obtained by choosing a random number between 1 and 5 (in this example it was 2). To calculate volume, the area of each slice must be estimated using *one* face only, e.g. the more occipital face (arrowheads). The distance between these slice planes is 5*d*. Notice that slice 27 has no occipital face and so cannot be analysed. Slices 1–26 could be used to estimate volume in the same way but the distance between slice planes would then be *d*.

MATERIALS AND METHODS

For the purposes of this sampling study, 15 fixed human brains were employed. For the MRI part of the study, one formalin-fixed specimen was used. All other brains were fixed by a standard embalming procedure used for dissecting room cadavers and described elsewhere (Henery & Mayhew, 1989). These brains, from an aged set of individuals, were sliced physically using a brain-knife. Before slicing, each brain was divided at the level of the superior colliculi in order to separate the forebrain (diencephalon plus telencephalon) from the remainder.

Cavalieri volume estimation

The volume of any arbitrary object can be obtained using the principle of Cavalieri (Gundersen & Jensen, 1987; Michel & Cruz-Orive, 1988). The following practical steps are necessary.

1. Take an exhaustive set of parallel sections through the object at a known mean distance between sections, *d* (see Fig. 1). This will generate a set of slabs of mean thickness, *d*. The location of the first section must be uniform random in the interval 0 to *d*. Section orientation is not critical to achieving unbiasedness but it may influence precision. Only one face of each slab (the most posterior, say) is analysed. Note that any end-slab which lacks such a face must be discarded.

2. Estimate the planar areas of the appropriate faces of the slabs by superimposing a systematic array of test points on each section. Given random positioning of the test

Table 1. *Worked numerical example to illustrate the unbiasedness of systematic sampling. An arbitrary object has been serially sectioned into* 24 *uniform random parallel slices. Slice areas are given in arbitrary units*

Slice number	Slice area	Slice number	Slice area
1	27	13	96
2	37	14	89
3	44	15	89
4	52	16	96
5	55	17	89
6	59	18	94
7	69	19	96
8	73	20	84
9	80	21	75
10	84	22	54
11	92	23	33
12	96	24	7

Total area = 1670

Sample with probability 1/4 and this will yield 4 possible samples, namely
Sample 1: $27+55+80+96+89+75 = 422$ ($\times 4 = 1688$)
Sample 2: $37+59+84+89+94+54 = 417$ ($\times 4 = 1668$)
Sample 3: $44+69+92+89+96+33 = 423$ ($\times 4 = 1692$)
Sample 4: $52+73+96+96+84+7 = 408$ ($\times 4 = 1632$)

Note the following: (1) each slice had the *same* chance of being chosen; (2) the mean of the samples is *exactly* 1670

array on each section, the total number of test points, ΣP, which falls on the sections affords an unbiased estimator of their total area, A. The exact relationship is $A = \Sigma P \times a(p)$, where $a(p)$ is the areal equivalent of one test point.

3. Estimate the volume of the object from the total area and the mean distance between slab faces

$$V = A \times d = \Sigma P \times a(p) \times d.$$

Note that this simple and efficient volume estimator requires absolutely no assumptions about object shape or spatial orientation.

In this investigation, sections of the cerebral hemispheres were generated by slicing noninvasively (using MRI) or with a brain-knife. Estimates of forebrain volumes and ventricular volumes were then estimated by point counting. For the brain sliced by MRI, its volume was estimated subsequently by fluid displacement. For physical slices it is known already that this volume (and brain weight) correlates extremely well with Cavalieri volume estimates (Henery & Mayhew, 1989; Mayhew *et al.* 1990).

MRI and Cavalieri estimation

All MRI slices were obtained using a General Electric 1·5T Signa MR unit. The head-coil chosen was designed for clinical imaging. The brain specimen was positioned centrally within the coil but, to ensure uniform random slicing, its position along the coil was randomised. In order to guarantee T1-weighted slice images, the pulse sequence was applied by an ordinary multiple echo (ME) technique with an echo time (TE) of 20 ms and a repetition time (TR) of 300–400 ms. This ME pulse sequence is comparable to that used in clinical studies on the anatomy of the brain, head and neck.

For all images, a field of view of 200 mm × 200 mm was applied, together with a

Table 2. *A set of* 6 *slices* (*see sample* 1 *in Table* 1) *is used to illustrate how to calculate the expected CE of the estimated total area* (*and hence volume*) *of the arbitrary object*

Slice number	Area, a	Product $a \times a$	Product $a \times (a+1)$	Product $a \times (a+2)$
1	27	27 × 27	27 × 55	27 × 80
5	55	55 × 55	55 × 80	55 × 96
9	80	80 × 80	80 × 96	80 × 89
13	96	96 × 96	96 × 89	96 × 75
17	89	89 × 89	89 × 75	
21	75	75 × 75		
Totals:	422	32916	28784	21760

Expected CE $= [(3 \times 32916 + 21760 - 4 \times 28784)/12]^{0.5}/422$
$= [(98784 + 21760 - 115136)/12]^{0.5}/422$
$= [5372/12]^{0.5}/422$
$= 21{\cdot}16/422$
$= 0{\cdot}0501$ (or 5·01 %)

quadratic matrix image of 256 × 256 pixels. For this coil, the signal-to-noise (S/N) ratio is heavily dependent on the field of view and on the volume within the coil (the 'fill factor'). The physical resolution was 0·7 mm.

Slice selection was performed within the interleave mode. This permitted selection of parallel systematic slices without any intervening spaces (i.e. the distance between slice centres is equal to the slice thickness). After a preliminary trial to see how image quality varied with slice thickness, it was decided to adopt a thickness of 5 mm with a 5 mm separation between successive slice centres (i.e. $d = 5$ mm). All slices were coronal sections as defined anatomically. A total of n = 28 MRI slices was generated and hardcopy negatives printed automatically at a final linear magnification of × 0·75.

To assure the best possible S/N ratio in the images with the selected pulse sequences and coil, 4 signals were averaged together to determine each distinct position-encoded signal in the final reconstructed image. Normal clinical procedure would be to choose one or two signals. The choice of 4 improved the S/N ratio in our final images by a factor of between 1·414 and 2.

The complete set of MRI slices provided a sample from which smaller subsamples could be drawn. Two different randomised sampling procedures were invoked: systematic and simple random sampling. In each case, slice images were analysed by point counting using a transparent overlay with a quadratic array of test points spaced at 1 cm. The point spacing was equivalent to a distance of 1·33 cm at × 0·75 with an areal equivalent of $a(p) = 1{\cdot}778$ cm^2. The overlay was randomly superimposed on each image in turn. As a supplement to the main purpose of the study, we also estimated the volume of the ventricles. Out of 28 slice images, 20 contained sections through ventricles.

Systematic sampling

Systematic selections of slices were drawn from the complete set. In order to monitor the effects of sample size and precision of estimation (expressed as coefficient of error, CE), various types of systematic selection were made. These gave individual slices probabilities of being picked equal to 1/2, 1/3, 1/4, 1/5 and 1/8. The

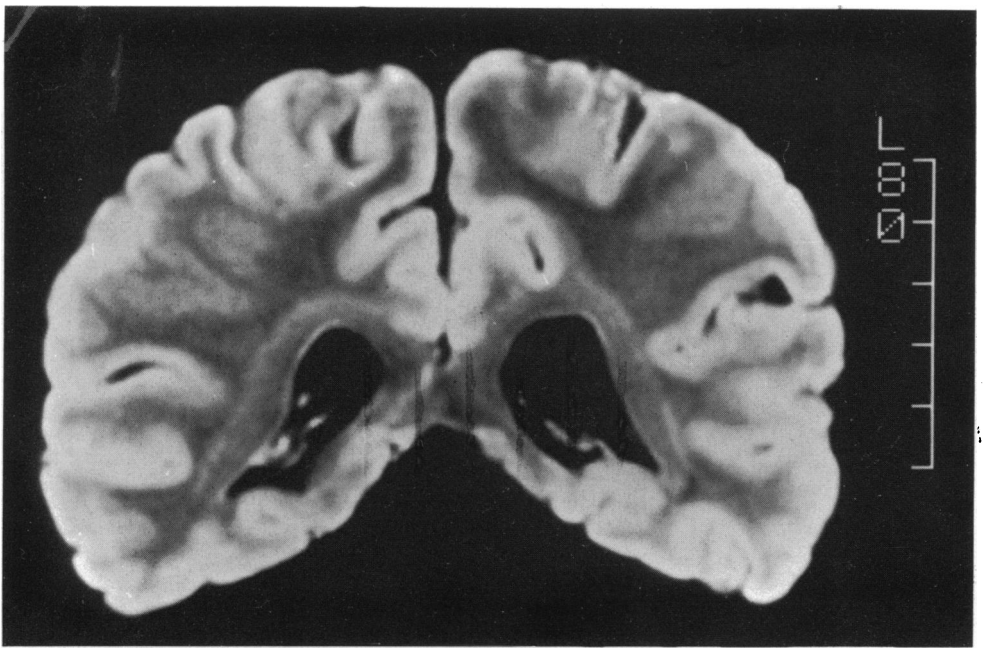

Fig.2. A coronal MRI slice through the forebrain showing the lateral ventricles. The scale to the right represents an actual length of 5 cm.

unbiasedness of the systematic selection principle is illustrated with a numerical example in Table 1. In that example, a sampling probability of 1/4 is adopted.

Simple random sampling

In order to compare the efficiencies of systematic versus simple random sampling, simple random selections of slices were drawn. To ensure strict comparability, the samples were drawn so as to correspond in size with the 1/5 systematic selections described above. In each case, the slice numbers (1–28 inclusive) were picked by lottery. This was equivalent to sampling without replacement.

Coefficient of error of volume estimates

The method for estimating CE for a set of systematic slices through an object is given in Gundersen & Jensen (1987, p. 237; Table 1). A worked example is offered in Table 2 using data provided for sample 1 in Table 1. The example is based on slice areas but the underlying equation applies equally well to the slice point totals which are used to compute those areas (see Michel & Cruz-Orive, 1988; Pakkenberg & Gundersen, 1988).

Brain weight and displacement volume

Brain weight was expressed to the nearest 10 g. Brain volume was determined using Archimedes' principle. The brain was divided by a sagittal cut through the corpus callosum and the volume of each hemisphere was determined separately by immersion in water in a measuring cylinder. The average of 2 estimates of total forebrain volume was taken.

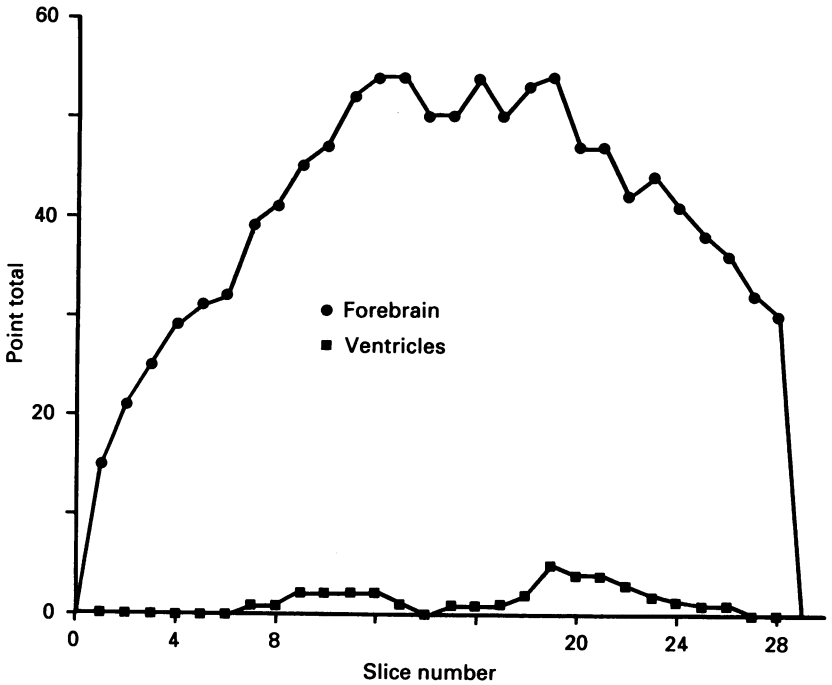

Fig. 3. The variation of slice area for 28 coronal slices through a human brain generated by MRI. Area is expressed as number of test points falling on total forebrain and on ventricles alone. The area under each line is proportional to total volume.

Table 3. *Estimated volumes and expected CE values for various systematic samples of MRI brain slices*

Type of sample	Number of slices	Sample mean (ml)	Coefficient of error, CE	Mean CE
1/2	14	1024	0·017	0·017
	14	1026	0·017	
1/3	10	1072	0·022	0·027
	9	992	0·029	
	9	1011	0·029	
1/4	7	996	0·038	0·037
	7	999	0·041	
	7	1052	0·035	
	7	1052	0·035	
1/5	6	1049	0·044	0·049
	6	1058	0·042	
	6	1098	0·038	
	5	973	0·058	
	5	947	0·058	
1/8	4	1052	0·081	0·097
	4	1116	0·072	
	4	1159	0·068	
	4	1138	0·071	
	3	939	0·117	
	3	882	0·114	
	3	946	0·119	
	3	967	0·112	

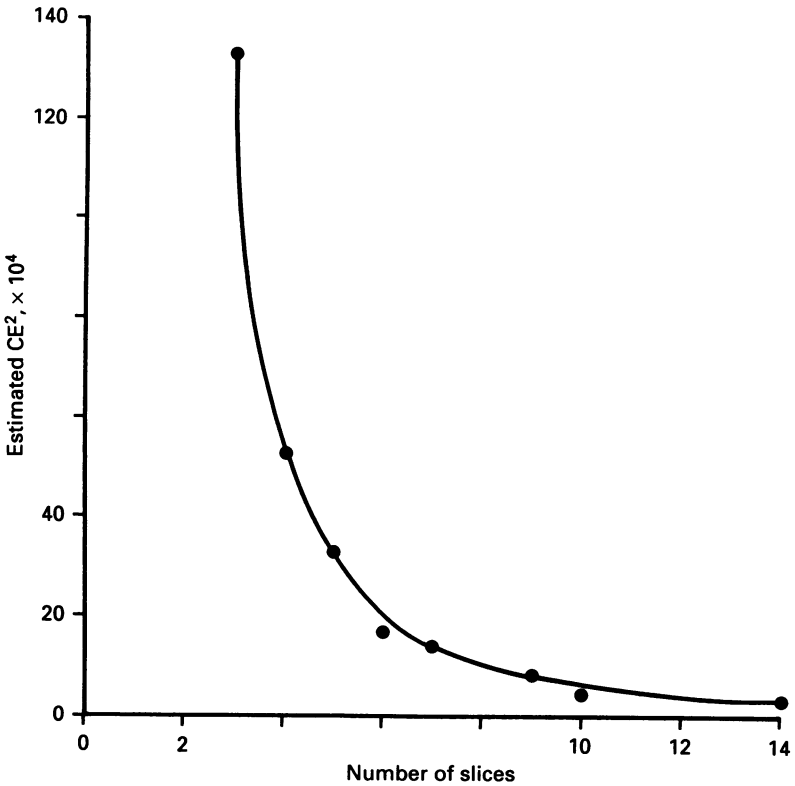

Fig. 4. Variation of expected relative sampling error (expressed as square of coefficient of error) with number of slices for Cavalieri estimates of cerebral volume. Each point represents either a single empirical estimate or the average of 2–4 estimates (see Table 3). The curve is close to that which would be expected if CE were proportional to $1/n$.

Observed coefficient of variation between brains

In order to assess the observed coefficient of variation (OCV) between brains, the 1/5 systematic sampling regime was applied to a set of 14 forebrains. The value of OCV provided a yardstick with which to assess the impact of the sampling precision expressed by CE. Both the real (natural or biological) variation between brains (RCV) and CE will contribute to the total OCV but, in a sensible experimental design, the CE value should be controlled to ensure that its contribution is less than that of RCV (see Shay, 1975; Gundersen & Østerby, 1980; Gupta *et al.* 1983; Gundersen, 1986).

RESULTS

MRI samples

A specimen MRI slice is illustrated in Figure 2. The areal profile for the complete set of 28 MRI slices through the forebrain is shown in Figure 3. For this set, the Cavalieri estimate of brain volume was 1025 ml and the expected CE was 0·009. Total ventricular volume amounted to 32 ml (with a CE of 0·038).

The estimated volumes and CEs obtained by the 5 systematic sampling regimes are summarised in Table 3. Taking a 1/2 sample, mean volume varied between 1024 ml and 1026 ml (CE 0·017 in both cases). Decreasing sample size (i.e. slice number) by

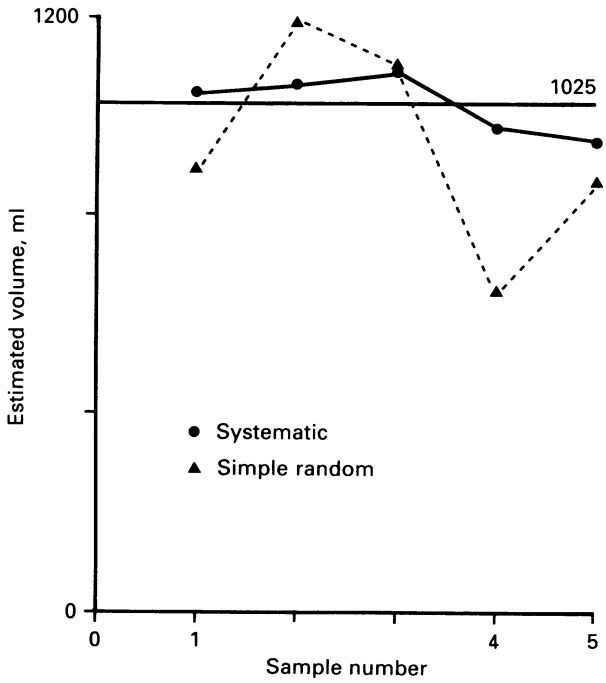

Fig. 5. Superior efficiency of systematic over simple random sampling. A 1/5 systematic sampling scheme yields 5 possible samples whose Cavalieri volume estimates are indicated (joined by solid lines). An equivalent simple random scheme generates volume estimates (joined by broken lines) which deviate more widely from the mean value. The mean volume for this forebrain is 1025 ml.

using a 1/8 scheme increased the range of estimated volumes (882–1159 ml) and their associated CE values (0·068–0·119; mean CE 0·097). The relationship between relative variance (expressed as CE^2) and slice number is illustrated in Figure 4.

In Figure 5, the estimated mean volumes are illustrated for a set of systematic samples (1/5) and for a set of simple random samples of comparable size (numbers of slices 6, 6, 6, 5 and 5 respectively). For the simple random samples, estimated mean volumes varied from 644 ml to 1187 ml, a much greater range than that provided by comparable systematic samples of slices (range 947–1098 ml and mean CE 0·049).

For comparison, the brain weighed 1090 g and the volume determined by fluid displacement was 1060 ml.

Brain-to-brain variability

For 14 brains physically sliced by the 1/5 systematic sampling scheme, the group mean Cavalieri volume was 796 ml with an OCV between brains of 0·15 (Table 4). Mean ventricular volume amounted to 30 ml (OCV 45%).

The OCV between total forebrain volumes represents a relative observed variance (OCV^2) of 0·0225. By way of contrast, the CE for the total set was only 0·009 and this represents a relative sampling variance (CE^2) of only 0·000081. These figures demonstrate that nearly all the observed variance between brains was due to natural (i.e. real) biological differences and that hardly any was due to the sampling imprecision of the method. If the latter was allowed to rise to 10% of the total observed variance, the expected sampling variance would have been 0·00225, a value which is 0·00225/0·000081 or 27·8 times larger than that actually achieved.

Table 4. *Group means and observed coefficients of variation (OCV) for estimates of forebrain and ventricular volumes made on physical slices*

Sex	Volume of forebrain (ml)	Volume of ventricles (ml)
F	800	25·8
F	851	12·0
F	622	22·7
F	821	50·3
F	773	41·6
F	622	38·1
F	729	29·0
M	860	38·2
M	744	29·0
M	1094	28·9
M	812	19·8
M	875	57·2
M	697	14·1
M	846	15·1
Mean	796	30·1
OCV	14·9%	45·1%

From the above figures, it is clear that forebrain volume is estimated far too precisely when using all 28 slices. However, the approximate number of slices required for a reasonably efficient estimate of forebrain volume can be calculated. It amounts to $28/(27{\cdot}8)^{0{\cdot}5}$ or 5·3. In other words, 5–6 slices would suffice and this corresponds nicely to the 1/5 systematic sampling scheme. In fact, it can be shown that any systematic scheme which selects more than 3 slices per set will satisfy the condition that RCV should exceed CE.

Corresponding calculations based on the OCV and CE estimates for ventricular volumes suggests, again, that 5–6 slices through ventricles would be sufficient. This is roughly the number which might be found in a set of 7–8 coronal slices through the entire forebrain.

DISCUSSION

This investigation has demonstrated that systematically sampled MRI slices through the human brain can be used to obtain unbiased estimates of brain volumes. Provided that the number of systematic slices per brain exceeds 3, then forebrain volume can be estimated very efficiently and the natural differences between brains will still make the major contribution to total observed variance. In fact, choosing 5–6 slices per brain yields a CE of the volume estimates of only 4–6%. This level of imprecision has but a very modest impact on the total observed variation between brains. To estimate forebrain volume and ventricular volume on the same slices, it would be prudent to increase the sample size to roughly 8 systematic slices per organ.

In an earlier study on brain volumes determined using the Cavalieri principle applied to physical slices, Regeur & Pakkenberg (1989) concluded that 4–5 slices would be sufficient to estimate ventricular volume alone. This figure is in good agreement with present findings. They also concluded that about 13 slices would be required to estimate cortical volume alone. In this study, cortical volumes were not estimated because one brain was not enough for us to be sure whether or not a resolution-dependent overprojection effect was present. The possibility of using MRI

slices for such information must therefore await further analyses on larger numbers of brains.

The question of how many points to apply to a given set of brain slices is one which has been answered already (Gundersen & Østerby 1980; Gundersen & Jensen, 1987). Between 100 and 200 test points per set of brain slices (to estimate forebrain volume) or per set of ventricle-containing slices (to estimate ventricular volume) would suffice. The finding that simple random sampling is less efficient than systematic sampling in the present context is a trivial confirmation of a general observation (Mayhew, 1983) which has been amply illustrated using biological examples by Gundersen & Jensen (1987).

The estimated volume of the MRI brain (1025 ml) is within the range of values found in this study (620–1095 ml) and in the literature (e.g. Blinkov & Glezer, 1968; Henery & Mayhew, 1989; Regeur & Pakkenberg, 1989). The group mean volume (800 ml) is lower than that observed in a set of Danish brains (1000 ml, see Regeur & Pakkenberg, 1989) but the OCV (about 15% of the group mean) is remarkably similar in both groups. There is slightly better agreement between the 2 groups for mean estimates of ventricular volume: 30 ml (OCV 45%) and 27 ml (OCV 37%), respectively.

The results for ventricular volume are consistent with the observations in the literature (cited in Regeur & Pakkenberg, 1989) that ventricles enlarge with age. Present results, based on aged brains, are at the higher end of the range of published values. Analysis of CT scans using the Cavalieri principle has shown that ventricular volume varies between 50 ml and 900 ml in the brains of hydrocephalic patients (Pakkenberg *et al.* 1989). In this context, ventricular volume affords a valuable means of monitoring the effects of shunting of cerebrospinal fluid. Ventricular enlargement is evident in the brains of patients with cerebral atrophy such as in Alzheimer's disease (see Ashtari *et al.* 1990) and is also detectable in schizophrenia (Pakkenberg, 1987).

The fact that brain and ventricular volumes can be estimated efficiently and unbiasedly by MRI has 2 important practical implications. First, the disadvantages of using model-based methods (which are, in general, never unbiased) can be avoided. Second, the undoubted statistical, diagnostic and predictive benefits of being able to conduct longitudinal studies on living subjects can be preserved. In future studies, it is intended to explore the possibility of estimating cortical volumes and surface areas from MRI slices using unbiased stereological methods (see Baddeley, Gundersen & Cruz-Orive, 1986; Henery & Mayhew, 1989; Regeur & Pakkenberg, 1989). Two main sources of technical bias might influence such estimates, namely the over-projection effect and inadequate resolution (Weibel, 1979; Gundersen & Jensen, 1987). The former is sensitive to image contrast and to slice thickness; the latter may hinder the clear definition of cortical surface at intrasulcal sites though not that which is visible on gyri. These biases do not occur when physical slices of mammalian brains are employed.

SUMMARY

A complete set of parallel (coronal) slices through a fixed human forebrain was generated by magnetic resonance imaging (MRI) and the Cavalieri principle, combined with point counting, was used to estimate brain volume. Alternative sampling schemes for estimating volume were then assessed by taking systematic and simple random selections of slices. Later, the brain was weighed and its fixed volume determined by fluid displacement.

For the complete set of $n = 28$ MRI slices, the volume (1025 ml) was estimated with a coefficient of error (CE) of less than 1%. Decreasing the number of slices by systematic sampling increased the CE but this was still only 5% when just 5–6 slices were analysed. Estimated volumes varied from 947 ml to 1098 ml. Simple random sampling was less efficient (estimated volumes for 5–6 slices were 644–1187 ml). The forebrain actually weighed 1090 g and displaced 1060 ml of fluid.

A set of 14 other brains was physically sliced in order to assess sampling errors in the context of observed brain-to-brain variation. It was found that 5–6 slices per brain is enough to yield efficient estimates of mean brain volume.

The findings demonstrate the practicability of using MRI to estimate brain volumes unbiasedly and efficiently. The methods have great potential for noninvasive, longitudinal studies on *in vivo* brains and other organs.

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