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The cerebrum and cerebellum of the fixed human brain: efficient and unbiased estimates of volumes and cortical surface areas*

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INTRODUCTION

The weight, volume, surface area and linear dimensions of the human brain have been studied from many and diverse aspects (for a review, see Blinkov & Glezer, 1968). Often these variables are estimated from specimens fixed either by immersion or by *in situ* perfusion. Weight can be determined quickly and accurately. Linear dimensions (length, height, width) can also be estimated rather easily using caliper measurements. Volumes may be calculated using fluid displacement or by cutting the brain into parallel slices and then estimating slice areas and the distance between slices (Blinkov & Glezer, 1968). If a properly randomised (systematic) sample of slices is selected, the latter method is tantamount to using the Cavalieri principle (Gundersen & Jensen, 1987). This principle produces extremely efficient and unbiased volume estimates for organs and their compartments (Gundersen & Jensen, 1987; Michel & Cruz-Orive, 1988; Pakkenberg & Gundersen, 1988).

No analogue of the Cavalieri principle is available for unbiased estimation of cortical surface areas from the perimeter lengths of parallel brain slices of known separation. Methods which attempt to estimate surfaces from such slices (Bok, 1939; Blinkov & Glezer, 1968) are biased by surface curvature effects which are minimised, but not eliminated, by reducing the slice interval. However, increasing the number of slices reduces efficiency. An alternative approach, i.e. covering the brain with thin foil which is subsequently removed and its area measured (see Blinkov & Glezer, 1968), is not only very laborious but also has the major disadvantage of failing to allow for cortical surface which is hidden in, say, sulci or the insula. Moreover, the method is clearly impracticable for cerebellar cortex which has a much smaller radius of surface curvature on its folia. A third approach, chemical impregnation of cortex with metal salts (e.g. Leboucq, 1926), is sensitive to variation in cortical thickness and not solely to surface area.

Fortunately, stereological methods for unbiased estimation of surface areas have been developed. For present purposes, the ideal candidate is the method of vertical sectioning (Baddeley, Gundersen & Cruz-Orive, 1986; Cruz-Orive & Hunziker, 1986; Mayhew, 1988) because its application can be combined conveniently and efficiently with Cavalieri estimates of brain volumes (Michel & Cruz-Orive, 1988). To date, however, this combination has not been used on the human brain so the impact of these techniques (one 3 years old, the other 300 years old) in this field is unknown.

In this report, sampling schemes are described for estimating volumes and cortical surface areas from macroscopic slices through fixed human brains. The schemes are

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Table 1. *Sex, age, cause of death and putative handedness of subjects employed in this study*

Subject number	Sex	Age (years)	Recorded cause of death	Dominant hand
1753	M	80	Bronchopneumonia; cancer of colon	Right
1755	M	77	Myocardial infarction	Right
1759	M	79	Acute heart failure; bronchitis	Not known
1762	M	79	Bronchopneumonia	Not known
1763	M	81	Bronchopneumonia	Not known
1764	M	76	Bronchopneumonia; chronic obstruction of airways	Not known
1750	F	76	Myocardial infarction	Right
1751	F	85	Myocardial infarction	Right
1756	F	70	Bronchial carcinoma	Right
1761	F	70	Heart failure; cancer of bladder	Not known
1765	F	98	Myocardial infarction	Not known
1768	F	88	Bronchopneumonia; cancer of breast	Not known

illustrated with examples from an analysis of sex and laterality differences in the cerebral hemispheres and cerebella from dissecting room cadavers.

MATERIALS AND METHODS

Provenance of brains employed

The brains were removed from cadavers embalmed by perfusion via femoral and radial arteries within 24 hours *post mortem*. The perfusate comprised ethanol (2840 ml), glycerine (550 ml), phenol (180 g), formalin (225 ml) and water (795 ml). Brains were not removed from cadavers until several months after perfusion, in accord with the dissection schedule employed in the department. Table 1 summarises the details of the age, sex and cause of death of the 12 subjects used. Equal numbers of males (average age 79 years) and females (average age 81 years) were taken. After removal, brains were stored in 70% (v/v) alcohol prior to examination.

Table 1 records also the handedness of the subjects where this was available. This volunteered information was incomplete but it can also be unreliable. To minimise possible confounding effects of handedness on the dimensions of right and left halves of the brain, we therefore tried to assess hand dominance by osteometry of the humeri from the same cadavers. It is known (e.g. Ruff & Jones, 1981) that human upper limb bones exhibit more pronounced right–left asymmetries than lower limb bones. From a pilot study on medical students, we confirmed that right–left asymmetries in humeral bicondylar distance offered a reasonable way of predicting the maximal incidence of left-hand dominance in the cadaveric samples. On cadavers (except 1755, for which bones were no longer available) we measured not only this distance but also humeral head size (maximum diameter). In every case but one (subject 1762, where distances were equal) the bicondylar distnce was greater on the right humerus. In 7 out of 11 cases, humeral head size was also greater in the right-hand limb. Finally, all subjects declared to be right-handed (Table 1) proved to have larger dimensions in the dominant limb. From these studies, we calculated that the upper limit for the number of left-handers in our sample was three. This is an overestimate because our calculations assume that the incidence of left-handed individuals in the population is 50%. The true value is probably between 3 and 30%.

Brain weight and linear dimensions

The forebrain was separated from the midbrain at a level just above the superior colliculi. The two cerebral hemispheres were then separated by a midline slice through the corpus callosum. The cerebellum was detached from the midbrain and the rest of the hindbrain by slicing through the peduncles as close to the cerebellum as possible. The cerebellum was then hemisected by a midline slice through the vermis.

Left and right cerebral hemispheres and left and right halves of the cerebellum were weighed to the nearest 10 mg on a Sartorius 2842 balance. The length of each hemisphere (distance between coronal tangents to frontal and occipital poles) was measured with calipers, as were width (greatest distance between sagittal tangents to medial aspect of hemisphere and temporal lobe) and height (greatest distance between tangents to lowest point on temporal lobe and highest point on medial border). For each half of the cerebellum, length was measured parallel to the brain stem axis and width was measured as the greatest lateral distance from the midline cut face.

Theoretical background to sampling

(*a*) *Vertical sectioning and cycloid test arcs*

A vertical section may be defined as any plane which is perpendicular to some arbitrary but identifiable reference plane. In order to estimate the surface area of any arbitrary object from vertical sections, certain sampling constraints with respect to section orientation and test lattice design must be met (for details, see Baddeley *et al.* 1986). The principal requirements are:

(1) The object (here, one cerebral hemisphere or half of the cerebellum) must possess a recognisable reference plane or one must be invented for it. This plane then becomes the 'horizontal' plane.

(2) Vertical sections must be cut perpendicular to the specified horizontal plane and the vertical axis must be recognisable on each of those sections subsequently.

(3) Vertical sections must be randomly orientated (isotropic) and randomly located on the horizontal plane.

(4) On vertical sections, intersections between the surface of interest and lattice test lines can be counted by superimposing cycloid test arcs (see Baddeley *et al.* 1986, their Figs. 3 and 7) which are aligned with respect to the vertical axis on each section. Intersection counts are then combined with point counts to derive an estimate of surface density within the volume of the object.

(*b*) *Cavalieri principle*

The volume of any arbitrary object can be obtained by the following sampling regime (see Gundersen & Jensen, 1987 and Michel & Cruz-Orive, 1988 for further details).

(1) Take an exhaustive set of parallel sections through the object. The sections can be equidistant but this is not crucial for unbiasedness provided that the *mean* distance between sections is known. Nor is section orientation critical, so any convenient orientation will suffice. However, section location must be randomised with respect to the object so that if the section separation is d cm then the location of the first section must be uniform random in the interval 0 to d cm.

(2) Estimate the planar areas of the systematic sections through the object. The most efficient way of achieving this is to superimpose uniform randomly on each

section plane a test lattice bearing a set of test points. Counting points which fall on each section plane through the object (or its compartments) provides an unbiased estimate of object (or compartment) area. From the areas and the interval between sections, an unbiased estimate of object volume is obtained.

Practical details of sampling

(*a*) *Cerebral hemispheres*

The medial aspect of each cerebral hemisphere was taken as a convenient 'horizontal' and all vertical sections were cut normal to this reference plane. For this purpose, each cerebral hemisphere was placed, medial aspect downwards, in a flat-bottomed enamel tray next to one side wall, i.e. that closest to the experimenter. On the inner side of this wall of the tray (chosen because it was not visible to the experimenter) was drawn a scale marked with equal (4 cm) intervals. The hemisphere was positioned blindly with respect to the scale so that the first slice (cut with a sharp brain knife) would be uniform random in the interval 0–4 cm. This initial systematic sampling generated coronal brain slabs (a set of 4–6 per hemisphere) which extended from frontal to occipital poles (Fig. 1*a–b*) and had a mean thickness of 4 cm.

The 4 cm interval was chosen deliberately in order to generate an average of about 5 slabs per hemisphere. In practice, these preliminary slabs do not need to be positioned uniform randomly. Because the volume and cortical surfaces of each slab were to be determined individually (see below), arbitrary positioning of slabs would be satisfactory. Here, uniform random cuts were made to satisfy sampling requirements for other variables not included in the present study.

Each slab was now cut into randomly orientated vertical slices by placing it roughly at the centre of a compass rose drawn on a transparent plastic sheet. The orientation of slices within the first slab of a set (the most frontal) was picked at random. The directions of slices in other slabs of the same set (Fig. 1*c*) were determined by systematically rotating successive slabs at approximately equi-angular increments of 180/N degrees where N is the number of slabs in the set.

These parallel vertical slices were cut systematically with the aid of a graduated scale marked at 3 cm intervals, the position of the first slice being uniform random with respect to each slab (Fig. 1*c*). By measuring the distance covered by these slices (excluding the two end-fragments), mean slice intervals could be confirmed for each brain (Gundersen & Jensen, 1987) but this confirmation was not necessary with the present scheme.

To facilitate later identification of the vertical direction consistently on *one* of the faces of each slice (note that using this convention, one of the end slices will be discarded; Fig. 1*c*), a 12 mm long metal staple was pressed gently into the white matter so that its long axis was aligned with the vertical. Between 10 and 12 vertical sections (slice planes) were obtained per hemisphere.

(*b*) *Cerebella*

Every cerebellum was hemisected by a roughly median slice through the vermis. The cut surface of each half-cerebellum was then identified as the horizontal and all vertical sections were taken normal to this reference plane. Essentially, the sampling protocol was a simple modification of that employed on the cerebrum. The principal differences were in the intervals between slabs (2 cm) and vertical slices (1 cm). These intervals were chosen simply because of the smaller size of cerebella. Altogether, 5–6 vertical sections were generated from each half cerebellum.

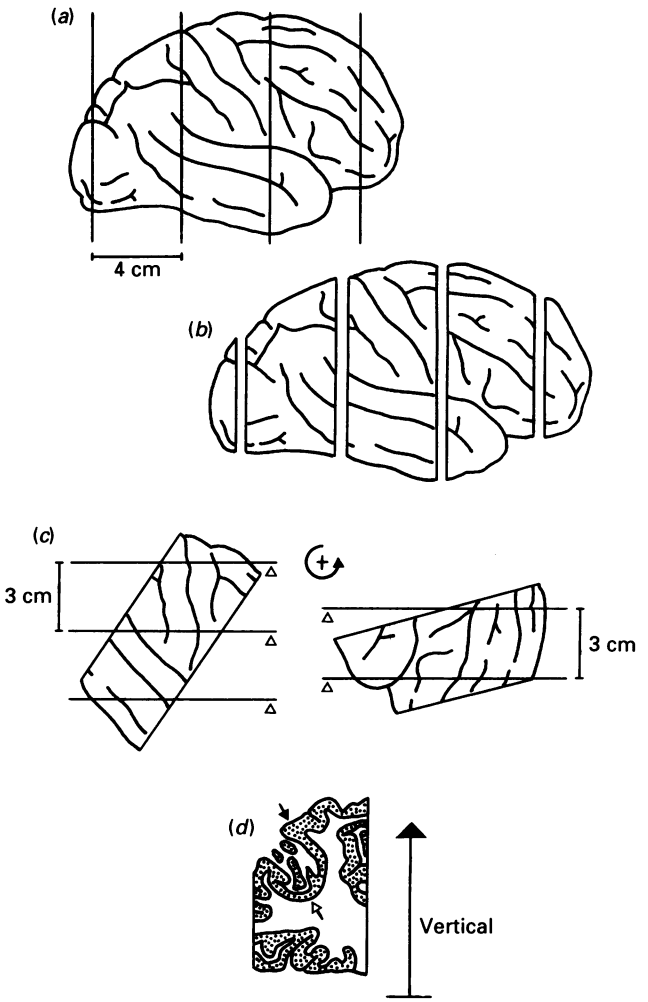

Fig. 1. Summary of sampling scheme for cerebral hemispheres. Each cerebral hemisphere is placed down on its medial surface (*a*) which provides a convenient reference 'horizontal' plane. It is then cut uniform randomly into parallel 4 cm thick slabs whose faces are normal to the horizontal (*b*). Every slab is then rotated systematically and cut, again perpendicular to the horizontal, into uniform random parallel slices of mean thickness 3 cm (*c*). Only *one* face of each of these vertical sections (e.g. that visible when viewed in the direction of the open arrowheads) is analysed. The cut surface of one such section is illustrated at (*d*) with the vertical direction shown. The vertical sections are used to obtain information about volumes and about outer (or pial, solid arrow) and inner (open arrow) cortical surface areas. A version of this scheme is used to sample each half-cerebellum.

Stereological estimations

The same lattice (bearing regularly arranged test points and cycloid test arcs) was employed to estimate volumes and surface areas. The lattice (see the staggered test lattice in Fig. 7*b* of Baddeley *et al.* 1986) had a test point area equivalent to 3·24 cm^2 on the specimen and a cycloid arc length equivalent to 1·29 cm.

(*a*) *Volumes*

The lattice was randomly positioned, but vertically orientated, consistently on one of the faces of each vertical slice in turn (Fig. 1*d*). The test points falling on all slices

were then summed and the total (P) used to estimate volume (V) by the Cavalieri relation, $V = Pad$ where a is the areal equivalent of each test point on the scale of the specimen (here, linear magnification was $\times 1$) and d is the estimated mean distance between slices (here, 3 cm for cerebrum and 1 cm for cerebellum). Slab volumes for cerebrum were estimated separately and then total volume calculated as the sum of the partial (slab) volumes.

As well as providing estimates of the volumes of each cerebral hemisphere and of each half-cerebellum, the same relationship was invoked to calculate the total volume of cerebral cortex. Where cortical definition was poor, resort could be made to staining by the Berlin blue modification of the Mulligan technique (Tompsett, 1970, pp. 217–219).

(*b*) *Surface areas*

Intersections between cycloid test arcs and the perimeter length of the cortical surface were summed over all vertical slices. Test points falling on slices were also counted. As required, repeat superimpositions of the lattice were made in order to achieve test intersection and test point totals of about 100 per hemisphere or per half-cerebellum (Gundersen & Jensen, 1987; Michel & Cruz-Orive, 1988). With two superimpositions per hemisphere, total intersection counts varied from 61 to 151 (average 99) and point counts from 61 to 114 (average 81). The corresponding figures for half-cerebella were 49–150 (average 103 intersections) and 10–28 (average 21 points).

The total number of intersections (I) was used to estimate the surface density of cortex within the reference volume (S/V) (whether cerebral hemisphere or cerebellum). The relationship is $S/V = 2I/(Pz)$ where P is the total of points falling on the reference space for the same set of lattice superimpositions and z is the length equivalent of a lattice point (i.e. the length of a cycloid arc).

To convert surface density (in cm^2/cm^3) into absolute surface (in cm^2), it was necessary to multiply by the appropriate reference volume (in cm^3).

These relationships were adopted to determine both outer (pial) and inner (white matter) surfaces of the cerebral cortex (Fig. 1*d*) so that cortical thicknesses could be estimated (see below). For cerebellum, only the outer cortical surface area was determined. For both cerebrum and cerebellum, the proportion of cortical surface hidden from view (within sulci, fissures, etc.) was also calculated from intersection counts.

(*c*) *Thicknesses*

The cerebral cortex may be envisaged as a much convoluted layer bounding the hemisphere. The thickness of this layer seems to fluctuate according to position (e.g. between gyrus and sulcus, between palaeocortex and neocortex and between regions of neocortex). Therefore, an estimate of global mean thickness must allow for these spatial differences. To accomplish this, the arithmetic mean thickness (T) of the entire cortex was estimated. This thickness represents the volume of cortex lying on a given surface. In fact, T was estimated for each hemisphere by dividing the volume of cerebral cortex by the mean of the outer and inner cortical surface areas.

(*d*) '*Shape*' *factors*

To test for isomorphy between left and right halves of the cerebrum and cerebellum of males and females, dimensionless coefficients (see Ross & Mayhew, 1984) were computed for each half-brain. These related cortical surface area, S, to cerebral or

cerebellar volume, V, as the coefficient $S^{1.5}/V$. For objects of the same configuration (and the same or different sizes), this coefficient is constant and so, in this narrow sense, it may be treated as a 'shape' factor in the present context.

(*e*) *Sectioning and analysis times*

To provide an indication of the ease of implementation of the Cavalieri and vertical sectioning protocols, the times taken for sectioning, for estimating hemisphere volume and for estimating the pial surface area of cerebral cortex were recorded separately. The times quoted reflect the range of values obtained for brains analysed at later stages of the study, i.e. when we were experienced in all aspects of the methodology.

Statistics

Values for each brain region were used to compute group means and standard errors (S.E.M.) for right and left sides of both males and females. The significance of apparent laterality differences was analysed by paired t tests for related samples, male and female brains being handled separately. This was done prior to applying two-way analyses of variance to test for any main effects of sex and laterality and for any interaction effect between these two factors (Sokal & Rohlf, 1981). The main purpose of this study was to sample for estimates of total volumes and outer cortical surface areas. Statistical data for other variables are provided for the sake of descriptive consistency and to aid in further interpreting the biological differences.

In a preliminary study to obtain empirical estimates of measuring errors, repeatedly counting 30–60 test points per hemisphere on the *same* slices indicated that the volume of a cerebral hemisphere was estimated with a coefficient of error (= S.E.M. × 100/mean) of less than 4%. For outer cortical surface areas, counting 30–80 test intersections per hemisphere, the comparable error was less than 7%. For each half-cerebellum, the equivalent measuring errors were 12% (volume) and 10% (cortical surface).

In this study, these measuring errors were reduced simply by superimposing lattices twice and thereby roughly doubling point and intersection totals. The overall errors due to stereology were not calculated because it has been shown before that the methods generate coefficients of error of 5% or less for as few as 5 sections per object (see Gundersen & Jensen, 1987; Michel & Cruz-Orive, 1988).

Total weights, volumes and surface areas per brain were obtained simply by combining estimates from the corresponding right and left halves. Relationships between total weights and volumes were examined using Pearson's product–moment correlation coefficients and linear regression analyses (Sokal & Rohlf, 1981).

RESULTS

Our findings for the right and left sides of male and female cerebra and cerebella are summarised in Tables 2–4. The time taken for sectioning a single cerebral hemisphere into vertical slices varied from 7 to 17 minutes. The total time for sectioning *and* estimating volumes and outer cortical surface areas came to 12–28 minutes depending, to some extent, on hemisphere size. Thus, total analysis time for one cerebral hemisphere amounted to less than 30 minutes. Total analysis time was less than this for each half-cerebellum.

Table 2. *Weights (g) and linear dimensions (cm) of cerebral hemispheres and cerebellar halves*

(Values are group means (S.E.M.).)

Variable	Males		Females	
	Right	Left	Right	Left
Cerebrum				
Weight	430 (19·3)	425 (14·7)	382 (22·5)	384 (20·8)
Length	15·5 (0·61)	15·9 (0·42)	14·3 (0·29)	14·5 (0·24)
Width	6·2 (0·74)	5·8 (0·64)	4·0 (0·15)	4·1 (0·17)
Height	7·0 (0·54)	7·1 (0·42)	6·6 (0·51)	6·7 (0·50)
Cerebellum				
Weight	51·6 (2·44)	51·2 (1·98)	47·9 (3·59)	48·5 (3·65)
Length	3·5 (0·08)	3·6 (0·07)	3·4 (0·08)	3·5 (0·09)
Width	3·3 (0·19)	3·1 (0·13)	3·0 (0·16)	3·0 (0·16)

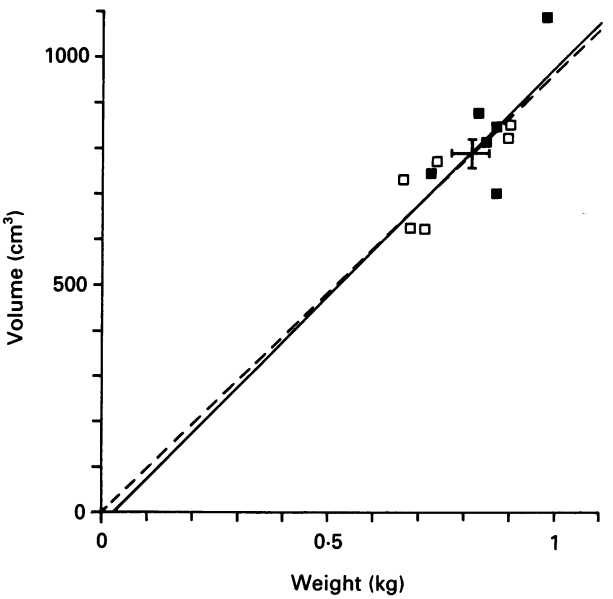

Fig. 2. Relation between weight and estimated volume of each cerebrum (both hemispheres combined). The cross with bars represents the common mean centre with its associated S.E.M. values. The solid line is the regression line and the broken line passes through the origin. Each square is one cerebrum (full squares are males; open squares are females). The correlation coefficient = 0·79 ($P < 0·01$).

Cerebral hemispheres

The average male hemisphere weighed 430 g and was significantly heavier than that in females (380 g, Table 2; sex difference $P < 0·05$ for 1, 20 D.F.). Apparent sex differences in Cavalieri estimates of hemisphere volumes (420 cm^3 *versus* 370 cm^3, Table 3) just failed to attain significance at the 5% probability level but there was a significant positive correlation between total cerebral (both hemispheres) weights and volumes ($r = 0·79$, $P < 0·01$ for 10 D.F.; Fig. 2).

Sexual dimorphism in hemisphere weight and volume seemed to be due to differences in lengths and widths rather than heights (Table 2) and was accompanied

Table 3. *Cerebral hemisphere volumes (cm^3), surface area (cm^2), thicknesses (mm) and shape factors (cm^3/cm^3) estimated stereologically*

(Values are group means (S.E.M.).)

	Males		Females	
Variable	Right	Left	Right	Left
Volumes				
Total	425 (38·8)	419 (31·4)	359 (27·9)	377 (27·0)
Cortical	157 (16·8)	161 (16·3)	141 (15·7)	159 (12·4)
Cortical surfaces				
Total	807 (80·1)	837 (66·2)	638 (48·8)	720 (49·6)
Hidden	534 (62·7)	547 (43·7)	447 (34·5)	505 (31·6)
Cortical thickness	2·22 (0·19)	2·11 (0·12)	2·36 (0·15)	2·49 (0·17)
Shape factor	54 (3·93)	59 (6·42)	46 (4·10)	52 (3·18)

Surfaces areas refer to pial aspect only.

Table 4. *Cerebellar volumes (cm^3), surface areas (cm^2) and shape factors (cm^3/cm^3) estimated stereologically*

(Values are group means (S.E.M.).)

	Males		Females	
Variable	Right	Left	Right	Left
Volume	35·1 (2·28)	34·0 (2·44)	30·8 (4·38)	32·7 (4·09)
Cortical surfaces				
Total	283 (17·2)	265 (26·4)	232 (29·3)	264 (36·2)
Hidden	239 (13·5)	230 (23·6)	202 (26·6)	231 (33·4)
Shape factor	136 (8·12)	131 (18·0)	116 (11·4)	130 (13·1)

by differences in cortical surface areas (820 cm^2 *versus* 680 cm^2, Table 3; $P < 0{\cdot}05$) but not cortical volumes (about 160 cm^3 in both sexes, Table 3). Approximately 66% of total cortical surface was obscured from view (Table 3).

The greater cortical surface in male cerebral hemispheres, coupled with lack of sexual dimorphism in cortical volume, suggests that the cortex is thicker in female hemispheres. As Table 3 shows, the arithmetic mean thickness of female cortex was about 2·4 mm compared to 2·2 mm in males. These findings also imply that the male and female hemispheres are not isomorphic. Apparently males have more cortical surface than might be anticipated simply on the basis of their greater volume (see shape factors, Table 3).

No significant lateral differences were detected in these samples. Nor were there found any significant interaction effects and this can be interpreted as indicating that sex differences, where present, applied equally to both hemispheres.

Cerebella

Despite apparent differences, no significant sex, lateral or interaction effects involving cerebella were found in these samples. On average, each half of the cerebellum weighed 50 g (Table 2) with a volume of 35 cm^3 and a cortical surface area of 260 cm^2 of which roughly 86% was hidden within fissures (Table 4). There was

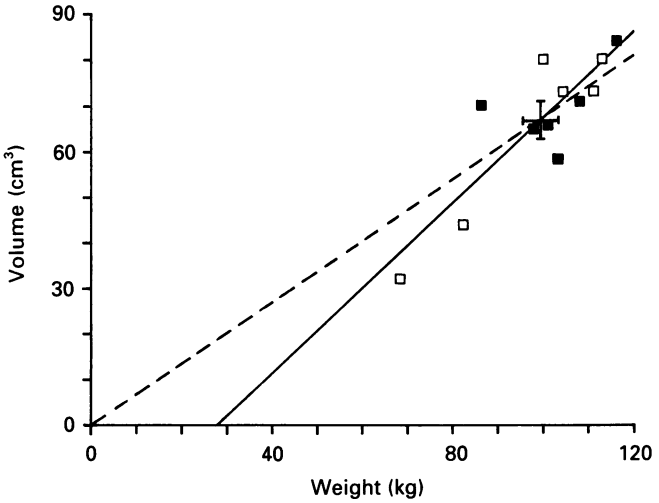

Fig. 3. Relation between weight and estimated volume of each cerebellum. The cross with bars is the common mean centre with its associated S.E.M. values. The solid line is the regression line and the broken line passes through the origin. Each square is one cerebellum (full squares are males; open squares are females). The correlation coefficient = 0·86 ($P < 0·001$).

a significant positive correlation between cerebellar weight and volume ($r = 0·86$, $P < 0·001$; Fig. 3).

DISCUSSION

The present study has demonstrated methods for obtaining unbiased estimates of brain volumes and surface areas quickly and easily by rigorous systematic random sampling into macroscopic slices. Significant sex differences were detected for cerebral hemispheres which tended to be bigger in males. No significant sex differences were found for cerebella. No evidence of lateral asymmetry in cerebral or cerebellar dimensions was detected. Sex effects, where present, affected both sides of the brain in the same way.

Appraisal of the methodology

Results are unbiased in the sense of being free from sampling and estimation bias (Mayhew, 1983) and valid for drawing internal comparisons. They are not unbiased in terms of technical errors, particularly those due to tissue distortions consequent upon processing procedures. It has been known for many years (see Blinkov & Glezer, 1968) that fixed brains suffer considerable shrinkage/swelling distortions. These effects are influenced by the mode of fixation and type of fixative but they are also age-dependent (Blinkov & Glezer, 1968; Haug, 1985). However, present findings relate to brains of similar ages fixed in a standardised fashion. Therefore, the statistical comparison of apparent sex and laterality differences retains its validity.

The Cavalieri method described here is based on a remarkably efficient and unbiased principle dating from the seventeenth century. Recent applications to a wide variety of tissues and organs (Gundersen & Jensen, 1987; Pakkenberg & Gundersen, 1988; Michel & Cruz-Orive, 1988) have demonstrated high efficiency selecting as few as 5–6 sections per object. Moreover, this can be achieved by point-counting without resort to mensuration devices (cf. Lange, Thörner, Hopf & Schröder, 1976). An

improvement in the design of this study would be to generate more test points per half-cerebellum (either by altering lattice size or by applying it more often) to reduce random errors. In general, estimator unbiasedness is conditional on section location being systematic random and so volume estimates are biased if this condition is not met (e.g. see Offord, Michiya, Oenning & Dyck, 1974).

The combination of Cavalieri and vertical sectioning not only preserves efficiency (less than 30 minutes to slice up a hemisphere and count 80 points and 100 intersections!) but also is the only sensible choice for estimating external surfaces of organs (see Michel & Cruz-Orive, 1988, for an application to the pleural surface of rabbit lung). Other stereological methods of surface estimation based on isotropic sectioning in three dimensions (such as the method of 'ortrips', Mattfeldt, Möbius & Mall, 1985) are unbiased but would be far less efficient for determining cortical surface areas. Earlier methods based on systematic sectioning have been biased by curvature effects and lack of randomisation of section location (Bok, 1939; Blinkov & Glezer, 1968) or by failure to account properly for anisotropy (Elias, Hennig & Schwartz, 1971).

Elias *et al.* (1971) attempted to cater for lack of isotropy in the coyote cerebral cortex by superimposing straight test lines on brain slices in three mutually perpendicular directions. For this purpose, one hemisphere was cut horizontally and the contralateral hemisphere was cut frontally. The same procedure was also used on one male and one female human brain (Elias, Kolodny & Schwartz, 1967). However, this procedure did not cater for anisotropy because (of course!) sagittal sections of a *third* hemisphere in the same brain could not be generated. An estimate of cortical surface area for a group of three whale brains was achieved by cutting the first horizontally, the second frontally and the third sagittally (Elias *et al.* 1967).

Comparison with earlier observations

Present estimates and conclusions are in broad agreement with those of earlier investigations but differ in detail (see Blinkov & Glezer, 1968; Lange *et al.* 1976). Clearly, the cerebral hemispheres employed here (weight of both; 770 g in females, 860 g in males) were lighter than those studied by others (1070–1290 g) and this would account for differences in volumes (740–840 cm^3 *versus* 920–1240 cm^3). However, our estimated surfaces (1360–1640 cm^2) are very close to previously published (but biased) morphometric values (1470–1670 cm^2) as is our estimate of the proportion of cortical surface hidden within infoldings (about two thirds, a fraction apparently attained between birth and two months). The surfaces are smaller than those determined by chemical methods (1500–2190 cm^2; Leboucq, 1926) and by biased sampling of brain slices (2200 cm^2 and 3050 cm^2; Elias *et al.* 1967). Cortical volumes (300–320 cm^3 or 40% of volume of both hemispheres) agree with earlier values (230–570 cm^3 or 44% of total volume).

Cerebellar weights (96–103 g *versus* 127–169 g) and volumes (64–69 cm^3 *versus* 130–162 cm^3) are also less than those recorded previously. However, cortical surface areas (500–550 cm^2 of which 86% is hidden within fissures) are in accord with published data (500–1200 cm^2 and 80–86% respectively).

Sex differences

Our findings confirm the tendency for male cerebral hemispheres to be heavier, more voluminous and possess a more extensive cortical surface than those of females. The greater size of the male brain was attributable to its greater length and width but not to any difference in height. Nor was sexual dimorphism in cortical surface

accompanied by significant differences in cortical volume. This implies that the female cortex is thicker, on average, than that in males and our findings seem to confirm this. Average thickness was 2·2 mm in males but 2·4 mm in females, both values somewhat smaller than the values of 2·9–3·0 mm reported elsewhere (Blinkov & Glezer, 1968; von Bonin, 1973). The sex differences in surface and volume of cerebral hemisphere and in cortical thickness also suggest that male and female brains are anisomorphic: the male hemisphere has more surface than might be anticipated simply on the basis of its greater volume.

Contrary to others, we have failed to demonstrate significant sexual dimorphism in the cerebellum and this might be due to the relatively small sample sizes used. Throughout most of human postnatal life, the absolute dimensions of female cerebella have been shown to be smaller than those of males although the ratio of cerebellar weight/brain weight seems to be the same in both sexes (Blinkov & Glezer, 1968). It may be that the smaller sizes of cerebella in this study partly reflect the ages of the subjects employed. It is known that cerebellar weights decline after 50 years of age and more or less equally in men and women.

Lateral asymmetries

As in other investigations, we have failed to demonstrate differences in volumes and surface areas between right and left sides of the cerebrum and cerebellum. This must not be taken to indicate that anatomical asymmetries between cerebral hemispheres do not exist. Studies on neurological and normal patients have shown repeatedly that right and left hemispheres display various functional asymmetries and these are accompanied by regional morphological asymmetries (for example, see Blinkov & Glezer, 1968; Geschwind & Levitsky, 1968; Wada, Clarke & Hamm, 1975; Kolb & Whishaw, 1980). Left–right asymmetries exist in the temporal speech cortex and may be manifest *in utero* by the second trimester. Other asymmetries exist in the length and slope of the lateral sulcus, in the length of the posterior horn of the lateral ventricle and in the upper region of the inferior frontal gyrus. The right (left) hemisphere may project further anteriorly (posteriorly) than the left (right) and thalamic noradrenergic neurons may show large lateral differences. In the rat, differences in cortical thickness have also been reported (Diamond, Johnson & Ingham, 1975).

Our failure to detect lateral differences in volume and surface area is not conclusive because cerebral hemisphere dominance is not complete. Enlarged cortical regions on one hemisphere may be offset by smaller areas elsewhere ipsilaterally or by larger areas of cortex in different regions contralaterally. To resolve these matters, investigations need to be undertaken using hemispheres divided further (into subcortical volumes and into different cortical regions). Finally, the question of laterality is also complicated by genetic and environmental confounders such as sex and handedness.

Concluding comments

Despite some overlap between present and previous estimates of cortical volumes and surface areas, the methodology described here offers advantages of unbiasedness and efficiency. The estimates are based on properly randomised sampling designs and unencumbered by unnecessary (and maybe invalid) assumptions about shape, curvature and orientation in space. Their true potential in human and comparative anatomical studies of normal and diseased brains (and many other organs) has yet to be realised. It is worth noting that slices for Cavalieri estimates of volume need not be confined to those cut with a knife, razor blade or microtome. Properly randomised slices generated from CAT and NMR scans are equally valid. Vertical sections could

also be produced by these scans but surface estimation (e.g. of intrasulcal cortex) might be biased by inadequate resolution.

With fixed material, shrinkage/processing effects distort the true volumes and surfaces but recent advances in stereology have eliminated the influences of these effects on estimates of neuron number (see Gundersen, 1986; Pakkenberg & Gundersen, 1988; Braendgaard, Evans, Howard & Gundersen, 1989; Nairn, Bedi & Mayhew, 1989). Whatever the fresh volume or surface of the human cerebellum in aged subjects, the total number of Purkinje cell nucleoli which it contains is 15 million on average (Nairn *et al.* 1989). In the case of human cerebral cortex, roughly 95% of its surface area represents neocortex and whatever neocortical surface (or volume) is in fresh brains, its total complement is about 14×10^9 neurons on average (Braendgaard *et al.* 1989).

SUMMARY

Extremely old and relatively new stereological methods for the efficient and unbiased estimation of volumes and surface areas were applied to fixed human brains. Brains from twelve subjects (six males aged 76–81 years, six females aged 70–98 years) were hemisected. Cerebral hemispheres and cerebellar halves from both sides were sliced systematic randomly for Cavalieri estimates of volume and vertical sectioning estimates of cortical surface area. Weights and linear dimensions were also recorded.

It took less than 30 minutes per cerebral hemisphere to estimate total volume and cortical surface area. Cerebellar halves were analysed even more quickly.

No significant differences between brain sides and no interaction effects were found but sex differences were confirmed. For male cerebrum (both hemispheres combined), the average volume was 840 cm^3 and cortical surface area was 1640 cm^2. Two thirds of this surface was hidden within sulci and in the insula. Cortical volume was 320 cm^2 with an arithmetic mean thickness of 2·2 mm. In females the cerebral hemisphere was smaller and the cortex was less extensive but just as voluminous.

In males, the cerebellum occupied 70 cm^3 with a cortical surface of 550 cm^2 of which 86% was hidden in fissures. Values were not significantly different from those found in females.

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