EFFICIENT AND UNBIASED ESTIMATION OF CEREBRAL VOLUME AND CORTICAL SURFACE AREAS OF GOAT BRAINS

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SUMMARY

Four brains from goats weighing between 10 & 40 kg were used to test an unbiased stereological method for estimating brain volume and cortical surface areas. The new method combines the Cavalieri method for estimation of volumes and the Vertical sectioning method for estimation of surface areas. Using a t-test no significant difference was found between left and right hemispheres; of cortical volume, inner and outer cortical surface areas, cortical thickness and shape factor. Neither was there any significant difference in weight between the left and right cerebral hemispheres. This method is quick and cheap (requiring very few and simple instruments) and gives unbiased estimates of surface areas. It is a very valuable method in Anatomy (e.g. testing for morphometric differences between sexes, for lateral asymmetries etc.); in Pathology (e.g. examining developmental and disease anomalies etc.) and in Toxicology (e.g. testing for alterations due to plant and chemical intoxication).

INTRODUCTION

Morphologic studies on the brain are easily done when dealing with weight, volume and linear dimensions. However, estimations of surface area or cortical volume and thickness are difficult and time consuming due to the numerous invaginations (sulci) and associated ridges (gyri) of the brain which make it impossible to easily trace the hidden surface.

As a result various methods have been developed to minimise error when estimating such parameters. One is the Cavalieri method recommended for unbiased estimation of volumes (Gundersen, H.J.G. & Jensen, E.B., 1987) and the Vertical Sectioning Method for unbiased estimation of surface areas (Baddeley et al, 1986).

A combination of the two methods has been used with success on human brains for unbiased estimation of both volumes and surface areas (Henery, C.C. & Mayhew, T.M., 1989). This new combined method is therefore being tested on goat brains to determine its effectiveness and practicability in the animal model.

MATERIALS AND METHODS

Four healthy goats, two males and two females were killed and their brains removed whole and thereafter left to stand in 4% formaldehyde. After proper fixation each brain was weighed and the cerebellum separated from the cerebrum. The cerebral hemispheres were separated from the brain stem at the level of the anterior colliculi. A clean midsagittal incision was made to divide the cerebrum into two halves. Further weighing was made

for the individual hemispheres and cerebellum. The medial face of each hemisphere was considered to be the horizontal (base and further cutting was made vertical to the horizontal plane to divide each hemisphere into three nearly equal slabs.

An initial angle was randomly chosen on a transparent plastic sheet on which a circle divided into 360° at 10° intervals had been drawn. One slab was placed on its own horizontal plane with one of its vertical faces parallel to the 0–180° line and then slices cut parallel to the line making the chosen angle (Fig.1). The distance between slice vertical faces on the same side was 7.5mm.

The face to be used for counting was determined by random selection and one end slice was therefore discarded because it had only one cut surface which was opposite to the one selected for counting.

Two lines, one vertical and one horizontal were drawn on a piece of paper making a 90° angle. The base (horizontal plane) of slice to be used in the count was placed parallel to the horizontal line on the paper and the face to be counted facing up. Counting was then done using a cycloid lattice (Baddeley et al., 1986 Fig. 7 b). This lattice on a transparent sheet is superimposed on to the face of the slice to be counted and points at which the arcs cross the outer or inner surfaces of cortex, counted. Similarly, cross points (+) which fall either on the cortex or anywhere on the slice are counted respectively. The length of the cycloid lines used in this case was 7.1mm. Each count was repeated three times on the same slice and totals for all counts in all slices obtained. When all slices from one slab had been used the second slab was cut but this time using an angle 60° larger than the previous. The 60° was obtained by 180/N where N = number of slabs in that hemisphere. At the end all pieces were used to measure the volume by displacement.

Calculations to determine volumes of each hemisphere, cortical volume for each hemisphere, outer and inner surface areas of cortex and cortical thickness were done according to formulae shown below.

Further calculations were done to determine total volume, surface areas, cortical thickness and shape factor as given:

Volume of hemisphere, $V_h = (P_t.d.a.)/n$; (n = number of times the count was repeated on each section only if such repetition was done).
Volume of cortex, $V_c = (V_h.P_c)/P_t$
Outer surface area of cortex, $S_o = (2.I_o.V_h)/(P_t.k)$
Inner surface area of cortex, $S_i = (S_o.I_i)/I_o$
Cortical thickness, $T_c = 2.V_c/(S_o + S_i)$
Shape factor $S = (S_o + S_i)^{3/2}/V_h$. This is an indicator of the degree of cortical folding, i.e. surface area regardless of brain size.

where:

- P_t = total number of points falling on all the sections
- P_c = number of points falling on the cortex alone
- I_o = number of cycloid arc intersections with outer surface of cortex
- I_i = number of cycloid arc intersections with inner surface of cortex.
- k = length of one cycloid arc (in this case k = 7.1 mm).
- d = distance between slice

vertical faces (in this case d = 10.0 mm).

a = area between 4 points (in this case a = 1.0 cm^2).

A Student t-test was used to test for symmetry for all parameters.

RESULTS

Counts for all four goats are shown in Table 1. These total counts were used to obtain the derived parameters in Table 1.

Table 1: Total counts on all sections for left (L) and right (R) cerebral hemispheres of each of the four goats.

	P_t	P_c	I_0	I_i	T_a (cm)	
Goat 1						
L		88	46	122	99	0.18
R		99	37	98	85	0.19
Goat 2						
L		123	77	148	117	0.15
R		124	70	172	127	0.16
Goat 3						
L		79	57	113	61	0.17
R		89	57	105	92	0.16
Goat 4						
L		99	47	112	94	0.16
R		104	62	110	104	0.10

Although there is a similar trend along columns for all goats e.g. goat no.1, L and goat no.2, L, there is a substantial range along rows e.g. the lowest value for volume of hemisphere, V_h for left hemisphere is 19.75 cm^3 and the highest is 30.75 cm^3. Similarly, mean differences between left and right sides (X_D L versus R) varied but there was no significant statistical difference as shown in Table 3.

It took between 30-40 minutes to make all physical analysis for each hemisphere.

DISCUSSION

Calculated cortical thicknesses were within 0.14cm - 0.23cm and direct physical measurements of the apparent thickness were within this range. The goats used were normal, healthy and as shown in Table 3 there were no statistical differences in any parameters between left and right hemispheres ($p > 0.05$). No attempt was made to compare the biologic parameters between sexes because of the small number of subjects per sex.

Table 2: Values calculated for individual hemispheres and total for goat brains

	Goat No.1			Goat No.2		
	L	R	Total	L	R	Total
$V_h(cm^3)$	22.00	24.75	47.75	30.75	31.00	61.75
$V_c(cm^3)$	11.50	9.25	21.00	19.25	17.5	36.75
$S_c(cm^2)$	85.92	69.01	154.92	104.2	121.1	225.32
$S_i(cm^2)$	69.72	59.86	129.58	82.39	89.44	171.83
$T_c(cm)$	0.15	0.14	0.15	0.21	0.17	0.19
$S^{3/2}/V_h$	88.29	59.11	100.50	82.89	98.56	128.17
$W_h(g)$	26.3	26.7	53.0	32.6	32.8	65.4
Vol.Shrinkage		%	38.0			18.3

	Goat No.3			Goat No.4		
	L	R	Total	L	R	Total
$V_h(cm^3)$	19.75	22.25	42.00	24.75	26.00	50.75
$V_c(cm^3)$	14.25	14.25	28.50	11.75	15.50	27.25
$S_c(cm^2)$	79.58	73.94	153.52	78.87	77.47	156.34
$S_i(cm^2)$	42.96	65.49	108.45	66.20	73.24	139.44
$T_c(cm)$	0.23	0.20	0.22	0.16	0.21	0.18
$S^{3/2}/V_h$	68.68	74.00	100.95	70.60	71.16	100.23
$W_h(g)$	24.10	23.20	44.30	27.40	27.00	54.40
Vol.Shrinkage		%	32.5			26.0

Using this method on human brains Henery and Mayhew (1989) could not find any difference between left and right cerebral hemispheres although they conceded that lateral differences could exist in association with functional regions with cancelling effects contralaterally as functional asymmetries are known to exist in humans.

Since this method proves to be simple, cheap and reliable it will be very useful and practical in investigation of developmental anomalies, infectious and non-infectious diseases affecting cortical volumes and surface areas as well as normal morphologic asymmetries; and differences between sexes and species. The method is unbiased because both sampling and counting are performed randomly resulting into minimum statistical error.

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Table 3: Results of paired t-test for difference between left and right hemisphere.

Parameter	$\bar{X}_D$ L vs R	SEM(X_D)	t (df=3)	P> 0.05
V_h	−1.688	0.5807	−2.906	NS
V_c	0.063	1.3593	0.0463	NS
S_v	1.77	7.041	0.2514	NS
S_i	−6.69	6.615	−1.0113	NS
T_i	0.0075	0.0202	0.3713	NS
$S^{3/2}/V_h$	1.91	9.6224	0.1985	NS
W_h	0.175	0.2955	0.5922	NS

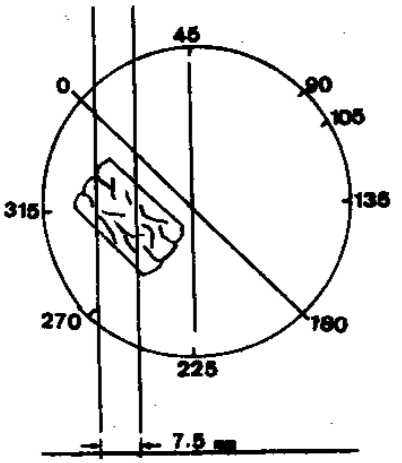

Figure 1: The circle is drawn on a transparent sheet and a slab placed on its horizontal plane, with its vertical plane parallel to the 0–180° line. Cutting into sections is done parallel to a line of a randomly chosen angle e.g. 45°. The next slat would be cut at an angle 60° larger, i.e. 150° in this case.

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