THE INFLUENCE OF AGE, LIVE WEIGHT AND GENDER ON THE MORPHOMETRICAL ASPECTS OF THE GOAT BRAIN DURING EARLY POSTNATAL DEVELOPMENT

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Abstract

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The study assessed the effects of age, gender and liver body mass on brain morphometric variables during the early postnatal life. The brains of 44 Florida Sevillana kids (22 female, 22 male) were processed using routine laboratory techniques in order to determine size (linear dimensions), weight and volume. The animals were analyzed at the age of 30, 45, 60 and 75 days.

Over the age-range studied, morphometric variables were found to increase. Analysis of covariance showed that in fact age only exerted a significant influence on brain weight and length; live weight, however, was the main factor of variation for all morphometric parameters except hemisphere width and height. Differences between sexes showed significantly greater influence of males than of females on brain weight, hemisphere weight and hemisphere length.

Brain morphometry, development, growth, width, height

Both brain morphometry and craniometry have been the subject of a great deal of controversy, particularly when used in research aimed at correlating brain size with alleged functional aspects.

Broadly speaking, the development of the mammalian brain shows that evolution has demanded a considerable increase in the surface area of the brain: the brain is known to develop from smooth surface vesicles which, in the course of growth, fold in upon themselves due to the spatial limits of the cranial cavity (Hofman 1985, 1989). Much more open to debate is the influence of individual factors, such as sex, age or body weight, on the brain growth (Mayhew et al. 1990, 1996).

This paper was prompted by an outstanding group of animals in terms of the great number of animals, the homogeneity of breed and the sequential grouping by age, even though it was limited to the early postnatal development. Kids belonged to a batch used for experimental carcass research by the University of Córdoba Animal Production Department, and were kindly donated by that department for present research purposes.

The principal aim of this study was to chart the evolution of the morfometric variables (weight, volume and linear dimensions) of goat brain during early postnatal development. A further essential aim of the study was to examine the correlation between these morphometric parameters and growth factors (age and weight) for each sex.

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Materials and Methods

This study was made using the brains of 44 Florida Sevillana kids (Herrera et al. 1991) from the Sevilla Provincial Council Animal Health and Production Service (Spain).

Kids (22 male, 22 female) were divided into four age- groups as follows:

group I. 8 females and 8 males. 30 days old.

group II. 4 females and 5 males. 45 days old.

group III. 7 females and 6 males. 60 days old.

group IV. 3 females and 3 males. 75 days old.

Table 1 shows live weight, carcass weight and head weight, by groups and as batch totals.

Following slaughter in an abattoir, brains were removed through a cranial opening and immediately weighed to the nearest decigram on an electronic balance. Brain volume was measured by liquid displacement, using a graduated cylinder containing an isotonic fluid.

Table 1

Data for live weight, carcass weight and head weight by group and for total batch divided by gender

	Live weight (g)			Carcass weight (g)			Head weight (g)		
Groups	Mean±SD	Max	Min	Mean±SD	Max	Min	Mean±SD	Max	Min
I	9306.3 (1582.5)	12300	7040	4878.8 (887.9)	6320	3400	507.1 (73.4)	616	398
II	11207.7 (1629.3)	13780	8760	5944.4 (828.8)	7400	4820	546.2 (71.4)	697	459
III	12646.2 (2113.2)	16920	9160	6461.5 (1142.8)	8680	4520	646.2 (121.1)	909	463
IV	13018.3 (2006.8)	15480	10460	6773.3 (1017.5)	7860	5340	660.3 (88.5)	761	557
Females	10338.1 (1739.0)	14380	7320	5396.2 (848.1)	6800	3840	534.0 (72.0)	653	398
Males	11964.3 (2588.7)	16920	7040	6212.2 (1375.0)	8680	3400	621.3 (126.5)	909	406

Brains were subsequently fixed in 10% formol, in which they remained for at least two months prior to processing. After this period, brain weight and volume were measured again to assess possible changes induced by the fixation process.

The following linear dimensions were then measured: length at pyramids (i.e. length from frontal pole to decussatio pyramidum), maximum width (maximum distance between temporal lobes of both hemispheres), and maximum height (maximum distance from the basilar surface to the dorsal surface).

The two cerebral hemispheres were separated by a midline slice through the corpus callosum. For each hemisphere, the forebrain was separated from the midbrain at the level of the colliculus rostralis. Only one of the two hemispheres was used for the present study, and was selected at random for processing; the other was kept in solution.

The weight, volume, length and width of each hemisphere were measured using the routine techniques described earlier. Hemisphere height was the same as the brain height already measured.

Statistics

Means and standard deviations for each variable were calculated using routine statistical procedures.

Variance was analyzed using a two-way analysis of variance (ANOVA) to test for the main effect of age and sex, and Scheffe's multiple range test to compare group means.

Given the interrelation of age and sex with body weight, an analysis of covariance (ANCOVA) was performed in order to ascertain the true influence of these factors on each variable. A simple ANCOVA model was used, taking regression with respect to live weight.

Finally, correlation coefficients between different variables and simple linear regression equations were calculated as a function of age and live weight.

In all comparisons, the null hypothesis was rejected at a level of significance of 0.05.

All statistical tests were performed using the General Linear Models Procedure of Statistical Analysis System (S.A.S.).

Results

Brain weight and volume were slightly modified by the fixation process. Modifications are shown in Table 2, which also indicates the shrinkage/swelling correction factor applied (i.e. the ratio of "fresh" data to "preserved" data). Figures for brain weight and volume in the remaining Tables are those obtained after fixation (i.e. after shrinkage/swelling).

Table 2
Effects of fixation on brain weight and brain volume, with corresponding corrector factors

	Brain Weight			Brain Volume		
Age groups	"fresh data"	"preserved data"	Correction factor	"fresh data"	"preserved data"	Correction factor
I	89.7	99.5	0.902	89.6	98.3	0.911
II	91.7	101.7	0.902	90.7	99.4	0.912
III	93.3	103.4	0.902	92.6	101.3	0.914
IV	100.5	107.7	0.93	102.6	106.6	0.962

Findings obtained for each variable by the total batch are summarized in Table 3.This table show means and standard deviations (in brackets) by sex and by group for each of the variables studied: brain weight (BW); hemisphere weight (HW); brain volume (BV); hemisphere volume (HV); brain length (BL); hemisphere length (HL); brain width (BWd); hemisphere width (HWd); brain/hemisphere height (HH). This Table highlights those cases in which ANOVA revealed significant differences due to age or sex; age-related differences are further broken down into specific inter-mean differences as revealed by Scheffe's multiple-range test.

Table 3
Mean and standard deviation (in brackets) for morphometric variables (all animals)

Variables	Females	Males	Total
BW (g)	99.4 (6.6) s	105.0 (8.8) a	102.3 (8.2)
HW (g)	38.9 (2.9) ss	41.3 (3.5) aa	40.1 (3.4) c
BV (cc)	97.4 (6.6) ss	103.7 (9.3) b	100.6 (8.6)
HV (cc)	38.3 (4.1)	40.0 (4.0)	39.1 (4.1)
BL (cm)	7.94 (0.23) ss	8.18 (0.35)	8.06 (0.31)
HL (cm)	6.75 (0.23)	6.85 (0.23)	6.80 (0.23)
BWd (cm)	5.86 (0.26)	6.00 (0.19)	5.93 (0.24)
HWd (cm)	3.00 (0.26)	3.07 (0.11)	3.03 (0.20)
HH (cm)	3.88 (0.24)	4.00 (0.24)	3.94 (0.25)

s, ss: Differences with respect to males $P \leq 0.05$ and $P \leq 0.01$, respectively
a, aa: By age groups and males, there are no differences between adjacent groups; differences between the rest $P \leq 0.05$ and $P \leq 0.01$, respectively
b: By age groups and males, there are no differences between groups I-II and III-IV; differences between the rest $P \leq 0.05$
c: By age groups, for total animals, group IV differs significantly from the rest $P \leq 0.05$

An analysis of covariance was performed to determine the effect of age, sex and live weight on morphometric variables, considering regression as a function of live weight. Sex-groups (male and female) and the age-groups described earlier were used for this purpose (Table 4).

The analysis of covariance revealed a clear influence of sex on brain weight, hemisphere weight, brain volume and brain length. An ANCOVA was therefore performed for each sex, in order to ascertain the effect of age by measuring regression with respect to live weight (Table 5).

Table 4
Analysis of covariance for variables studied as a function of age, sex and live weight

Morphometric variables	Variation factor F values		
	Age	Sex	Live weight
BW	2.86*	9.10**	25.50**
HW	1.21	4.66*	5.75*
BV	2.28	9.32**	16.98***
HV	0.36	2.58	16.52***
BL	2.91*	11.11**	8.12**
HL	1.79	3.52	31.48***
BWd	0.43	1.51	1.29
HWd	1.61	0.16	1.35

Regression considered as a function of live weight.
*, **, ***: F values statistically significant for P≤0.05, P≤0.01 and P≤0.001, respectively

Table 5
Analysis of covariance for variables for which intersex differences were found

	Females F values		Males F values	
Morphometric Variables	Age	Live weight	Age	Live weight
BW	0.21	2.82	7.99**	20.10***
BW	0.85	0.96	10.53***	14.56**
HV	0.36	0.42	7.31**	17.02***
BL	0.35	4.14	2.89	1.94

, *: F values statisticaly significant for P≤0.01 and P≤0.001, respectively

The following conclusions can be drawn from the results obtained:
1. Live weight was the main source of variation for practically all the variables studied.
2. Age exerted a significant effect on brain weight and length.
3. Sex mainly affected brain weight, hemisphere weight, brain volume and brain length. Separate analyses of covariance traced these effects - with the exception of brain length - to males.

Only hemisphere width and height appeared not to be influenced by any of the three factors (weight, age and sex).

The present study also recorded carcass weight and head weight. Since these three weight parameters are closely inter-related (Table 6), live weight was treated as influencing factor; the effects of this factor on brain growth are equally applicable to carcass weight and head weight. Correlation coefficients for total variables studied are also shown in Table 6.

Table 6
Correlation coefficients for total variables studied

	AGE										
Live W	0.65***	Live W									
Carcass W	0.61***	0.98***	Carcass W								
Head W	0.58***	0.93***	0.90***	Head W							
BW	0.32*	0.69***	0.72***	0.71***	BW						
HW	0.35*	0.69***	0.72***	0.70***	0.96***	HW					
BV	0.29	0.62***	0.66***	0.64***	0.96***	0.92***	BV				
HV	–0.04	0.39**	0.39**	0.44**	0.69***	0.66***	0.67***	HV			
BL	0.36*	0.61***	0.62***	0.60***	0.62***	0.58***	0.60***	0.48***	BL		
HL	0.25	0.67***	0.67***	0.66***	0.76***	0.75***	0.72***	0.61***	0.56***	HL	
HWd	0.11	0.29	0.29	0.24	0.35*	0.36*	0.21	0.28	0.32*	0.22	HWd
HH	–0.13	0.005	0.07	0.04	0.23	0.22	0.24	0.24	–0.03	0.23	–0.04

*, **, ***: F values statistically significant for P ≤ 0.05, P ≤ 0.01 and P ≤ 0.001, respectively

Various regression models were tested; those which best fitted the results of the present experiment were simple linear regressions (Table 7).

In the case of age, significant regressions were recorded for brain weight (BW), brain length (BL).

Table 7
Simple linear regression analysis

	Age		Live weight	
Variables	A	B	A	B
BW	94.58 ± 3.74	0.16 ± 0.07*	75.26 ± 4.51	$0.002 \pm 4 \cdot 10^{-4}$***
HW	37.41 ± 2.97	0.04 ± 0.06	28.11 ± 4.36	$0.001 \pm 4 \cdot 10^{-4}$*
BV	93.35 ± 3.99	0.15 ± 0.08	74.89 ± 5.14	$0.002 \pm 5 \cdot 10^{-4}$***
HV	39.65 ± 1.95	−0.01 ± 0.04	31.50 ± 2.83	$7 \cdot 10^{-4} \pm 2 \cdot 10^{-4}$**
BL	7.72 ± 0.14	0.007 ± 0.003*	7.16 ± 0.18	$8 \cdot 10^{-5} \pm 2 \cdot 10^{-5}$***
HL	6.63 ± 0.11	0.003 ± 0.002	6.06 ± 0.13	$7 \cdot 10^{-5} \pm 1 \cdot 10^{-5}$***
HWd	2.97 ± 0.09	0.001 ± 0.002	2.77 ± 0.14	$2 \cdot 10^{-5} \pm 1 \cdot 10^{-5}$
HH	4.24 ± 0.27	−0.005 ± 0.005	3.88 ± 0.42	$1 \cdot 10^{-5} \pm 4 \cdot 10^{-5}$

A, distance at the origin of the line of regression
B, slope of the line of regression
*, **, ***: F values statistically significant for $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

All morphometric variables showed significant or highly significant regression for live weight, with the exception of hemisphere width and height.

Discussion

Influence of fixation on morphometric variables

Brain measurements are modified by the fixation process. A number of authors have reported that formol causes shrinkage of tissues (Bauchot 1967; Sass 1982; Uylings et al. 1986). Nevertheless, morphometric data always refer to fixed brains, due to the practical difficulties involved in handling "fresh" brains.

In the present study, and as evidenced by the correction factors shown in Table 2, shrinkage through fixation was negligible. These correctors could, if required, be used to relate morphometrical data to live-brain data.

Influence of age and live weight on brain growth

One of the main purposes of this study was to correlate brain growth, in morphometric terms, with the general growth of the animal. To that end, experimental animals were grouped sequentially by age, in order to chart the effects of age on morphometric development of the brain. Live weight, one of the main indicators of general somatic growth, was also recorded.

There is evidently a close relationship between age and body weight during growth; increase in weight with age is so significant that it is generally used as a measure of the normality of growth. It would consequently appear reasonable to assume that age and body weight would prompt similar variations in morphometric data. This would undoubtedly have been the case if the age groups studied had been separated by intervals sufficiently wide to ensure a similar differentiation in live weight distribution between age groups. However, in the present study the interval between age-groups was so narrow that there was no clearly differentiated distribution of live

weight. This is seen in the oscillation of body weight (within normal levels) shown in Table 1, although body weight increases progressively with age, there is some overlap of ranges for each group.

This may account for potential interference between factors: the effects of one factor (e.g. age) may increase, mask or cancel out the effects of another (e.g. live weight). It is thus necessary to identify these effects in order to ascertain the real influence of each factor on growth.

Discriminant analysis of age and live weight as sources of variation in brain development yielded some interesting results. Although both factors are closely related, they are open to differring interpretations. Age represents a chronological development closely linked to the maturation of brain structures and circuits. At the same time, body weight represents somatic growth, of which brain growth is equally a part.

Since results here were expressed in terms of age groups, age was taken as the factor of variation for the first analysis of variance (Table 3); Results of analysis of covariance for variables studied as a function of age, sex and live weight (Table 4) showed that in fact age only exerted a significant influence on brain weight and length. Weight, however, was found to influence brain weight ($P < 0.001$), hemisphere weight ($P < 0.05$), brain volume ($P < 0.001$), hemisphere volume ($P < 0.001$), brain length ($P < 0.01$) and hemisphere length ($P < 0.001$). Weight was thus the main factor of variation for all morphometric parameters except hemisphere width and height. Variations in brain weight and length were attributable both to live weight and age. F values for age and live weight express their relative importance: 2.86/25.50 for brain weight and 2.91/8.12 for brain length. Live weight thus exerts greater influence than age, the difference being almost ninefold in the case of brain weight and threefold in that of brain length.

As Table 6 shows, carcass weight and head weight were both closely related to live weight. The effect of live weight as a factor influencing variations in brain morphometry is therefore also applicable to carcass weight and head weight.

Results of ANCOVAs, correlations and regressions suggest that, irrespective of live weight, increasing age is accompanied by increasing brain weight and, to a much lesser extend, brain length. This correlates with the need for cerebral structures to develop and mature with age, regardless of the space available, which is fundamentally determined by live weight.

Influence of gender on brain growth

Differences between sexes were highlighted by both the analysis of variance (Table 3) and that of covariance (Table 4) performed. Both analyses for the total number of experimental animals showed significantly greater influence of males than of females on brain weight, hemisphere weight and hemisphere length.

The ANCOVA performed to separate the effect of gender from the effect of live weight yielded similar results, suggesting that the interaction between sex and live weight was negligible.

ANCOVAs were performed for each gender in order to identify the factors giving rise to the differences mentioned above (Table 5). The results showed a highly significant effect of age and live weight on brain weight, hemisphere weight and hemisphere volume, but only in males.The strong influence of age, and particularly of live weight, recorded in males only is striking. Possible genetic or hormonal causes may be adduced to account for the fact that inter-sex differences for these variables are determined by males. However, a reasoned explanation of the causes, rather than mere speculation on a number of possibilities, lies beyond the scope of this paper.

Vliv věku, živé hmotnosti a pohlaví na morfometrické aspekty mozku koz během raného postnatálního vývoje

Mozky 44 floridských sevillských kůzlat (22 samičích, 22 samčích), rozdělených do čtyř věkových skupin (30, 45, 60 a 75 dnů), byly zpracovány rutinními laboratorními metodami pro stanovení velikosti (lineární dimenze), hmotnosti a objemu. Ve studii byl sledován také vliv věku, pohlaví a živé hmotnosti na celkové morfometrické změny mozku v raném postnatálním údobí. Studie ukázala, že se morfometrické změny zvětšovaly s věkem. Analýzou kovariance bylo doloženo, že věk významně ovlivnil pouze hmotnost a délku mozku; živá hmotnost však byla hlavním faktorem proměnlivosti všech morfometrických parametrů kromě šířky a výšky hemisféry. U kozlíků byly zaznamenány významně vetší rozdíly ve hmotnosti mozku, v hmotnosti a délce hemisféry než u koziček.

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