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Study of Possible Correlations between Body Weights and Brain Parameters in Neonatal and Mature Rats

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Abstract. Correlations between body weight, cerebral weight, cerebral DNA (cell number) and cerebral protein have been studied in a population of 249 neonatal (29 litters) and 107 adult normal rats. It was found that, on a statistical basis, an individual with a heavier neonatal body weight is also likely to have a higher cerebral weight, neonatal cerebral DNA (cell number) and neonatal cerebral protein. For a sample of this size, each pair of these parameters was significantly correlated. In adult animals the significance of correlations with body weights disappears, but the correlations between each pair of cerebral parameters remain significant.

Key Words
Correlations, body weights–brain
Brain parameters
Neonatal rats, brain
Body weights, neonatal
DNA, cerebral
Protein, cerebral
Rats, brain

The demonstration of the statistically significant correlations between brain parameters and neonatal body weight appears to be of importance: in humans and other animals studied, neonatal body weight is significantly correlated with placental weight [14, 16; reviews in 8, 20] and with the nutritional status of the mother [11, 19, 21]; body weight is often the only parameter that is routinely available at birth. Thus, the foresaid correlations, if established, will make it possible to estimate, on the basis of neonatal body weights, the concomitant neonatal brain development; the estimate would be statistically valid for a population, though it may not be valid in individual cases.

In this paper we present an attempt to establish such correlations in a large rat population.

Materials and Methods

Albino rats used in the previous [9–11, 17–21] and present works are Sprague-Dawley derived; these animals have now been bred in our laboratory for 34 generations. Virgin females 3 months old an weighing 200–260 g were mated. The animals were fed *ad libitum* a pelleted diet (Wayne Mousebreeder Block, Allied Mills, Chicago) containing 20.5% protein.

The animals were born between 1971 and 1973; in this study only those litters were used that were normal size (6–13, mean 8.86), had no dead newborns, had normal birth and were all analyzed for brain parameters (see below). Altogether 29 litters with 249 newborns were used.

The newborns were weighed and decapitated within 6 h after delivery. The brains (cerebral hemispheres, without cerebellum and olfactory lobes) were immediately removed and weighed; they were then frozen and subsequently used for analysis. DNA was determined by a modification of the diphenylamine colorimetric method [13, 15]; protein was determined by a modification of the LOWRY *et al.* [7] colorimetric method.

For the study of mature animals, newborns from additional litters were nursed by their mothers, weaned at 25 days and maintained on the same pelleted diet until the age of 196–340 days. They were then decapitated, the brains immediately removed and analyzed as described above.

To address the question of correlation among the four parameters, we computed their correlation coefficients within each of the samples. Submitting each of the computed values to a significance test, we found that some were significant, and some were not. Because of the small samples (the litter sizes, ranging from 6–13) involved, only very large values are judged significant. Among a large group of samples, if the actual population correlation is zero, one would expect to find about 5% of the observed coefficients statistically significant at the 5-percent level. Observation of more than this percentage among several significant samples is evidence that the population correlation coefficient is not zero. We thus applied the test for the parameter p of a binomial distribution to the observed percentage of significant values.

Results and Discussions

Table I lists the mean values ± standard deviations for all parameters and ages studied. Table II shows the correlations between body weight, cerebral weight, cerebral DNA, and cerebral protein for each of the 29 litters of neonatal animals. In each case several litters show statistically significant correlations, significantly more litters than the 5% or 1% which would be expected due to chance alone. Previously, such correlation was not observed [17], but since significant results appear sufficiently frequently among the 29 litters, we may conclude that all four of the parameters are correlated.

Table I. Mean values ± SD of parameters for neonatal and adult rats. Pooled samples

	Neonates	Adults	
		males	females
Postnatal age, days	0	340.6 ± 105.2	195.9 ± 58.7
Number of samples	249	15	92
Body weight, g	6.00 ± 0.413	425.9 ± 71.0	266.0 ± 39.0
Cerebral weight, g without olfactory lobe and cerebellum	0.160 ± 0.008	1.274 ± 0.055	1.196 ± 0.056
Cerebral DNA, μg	592 ± 26.2	1,079 ± 33.2	1,040 ± 98.2
Cerebral protein, mg	8.53 ± 0.87	100.2 ± 6.4	94.17 ± 8.57
Cerebral weight/cerebral DNA	272 ± 13.1	1,181 ± 50	1,160 ± 100
Cerebral protein/cerebral DNA	14.4 ± 1.41	92.94 ± 6.0	90.72 ± 5.52

To determine whether the variation in the observed correlation coefficients among litters could be explained as sampling variability, we computed Fisher's z statistic [5] for each of the correlation values, then obtained a probit plot. This plot turned out to be nearly linear, implying that the z values were approximately normally distributed as one would expect them to be if sampling variability were the sole cause of differences among them. This was done separately for each of the six correlation coefficients, with similar results in each case. Pooling of the litters to obtain more precise estimates of the correlations was therefore justified. The correlations between neonatal body weights and neonatal cerebral parameters, weight, DNA (cell number) and protein, for all litters pooled together, are presented in table III. It can be seen that for the sample of this size, *all* the parameters are significantly correlated [compare also 4, 14, 17]. It thus appears that, on a statistical basis, an individual animal with a higher neonatal body weight is also likely to have a higher neonatal cerebral weight, cerebral DNA (indicative of cell number) and cerebral protein (indicative of cell size). Since these correlations are for neonatal rats, the 'cell number' refers mostly to neurons (neuroblasts) [1]; the latter in rat cerebrum essentially do not multiply after birth [reviews in 17, 20]. Thus, the above results suggest that, within a large group of animals, the one with higher neonatal body weight is indeed more likely to have a higher *final* number of cerebral neurons. Since specific gravity of cerebrum is essentially constant, cerebral weight is proportional to cerebral volume. Thus, the ratio cerebral weight/cerebral DNA (cell number) is proportional to the volume of the cerebrum per one cerebral

Table II. Correlations between neonatal parameters [5, p. 569]

Litter	Litter size	B–C	B–D	B–P	C–D	C–P	D–P
1	9	0.8567**	0.3716	0.0893	0.4423	–0.1051	0.2820
2	9	0.6857*	0.5999*	0.3762	0.5482	0.7989**	0.4050
3	9	0.9389**	0.8559**	0.3757	0.8513**	0.2884	–0.0058
4	9	0.5344	0.7003*	0.5922*	0.8849**	0.8164**	0.7808**
5	6	–0.4101	0.8311*	0.4261	–0.5460	–0.6987	0.7200
6	8	0.7534*	0.5072	0.5228	0.3614	0.8697**	0.4828
7	11	0.8717**	0.5117	0.6552*	0.6910**	0.6396*	0.8427**
8	8	–0.2118	–0.3465	0.1621	0.7633*	0.5655	0.0748
9	8	0.9423**	0.6683*	–0.4934	0.8105**	–0.3402	0.0785
10	8	0.5535	0.1690	–0.1698	0.8145**	0.3145	0.0328
11	11	0.8569**	0.7431**	0.1968	0.9181**	0.3628	0.3676
12	10	0.7094*	0.7016*	0.6144*	0.6414*	0.2404	0.6063*
13	7	0.4571	0.1818	–0.4249	0.1501	–0.1824	–0.4964
14	8	0.6815*	–0.0039	0.3800	0.4045	0.2118	0.0277
15	9	0.5435	0.6111	0.4480	0.6282	0.1210	0.3294
16	9	0.8189**	0.6753*	0.3450	0.8235**	0.5425	0.6993*
17	6	0.8369*	0.3334	0.6185	0.1534	0.5335	0.9045**
18	8	0.7757*	–0.0225	–0.0913	0.4235	0.3149	0.9465**
19	13	0.4983*	0.4835*	0.2714	0.7461**	0.8031**	0.8088**
20	8	0.9339**	0.7411*	0.5189	0.6875*	0.5118	0.1139
21	8	0.9532**	0.6602*	0.6991*	0.8171**	0.8097**	0.7544*
22	11	0.1977	–0.1859	0.2126	0.5418*	0.7271**	0.3583
23	10	0.1704	0.4149	–0.0002	0.6487*	0.6196*	0.2236
24	7	0.9652**	0.8584**	0.8964**	0.8796**	0.8548**	0.7300*
25	8	0.9659**	0.1709	–0.1082	0.1016	–0.2598	0.1706
26	7	0.8279*	0.1720	0.3246	0.3219	0.5993	–0.3934
27	6	0.5780	0.4343	–0.0875	–0.4631	0.3694	–0.3643
28	11	0.5648*	0.0983	0.3707	0.3537	0.5198	0.4770
29	7	0.7231*	0.0855	0.8478**	0.6905*	0.9465**	0.4382
Number significant							
5-percent level		20**	12**	6**	16**	10**	9**
1-percent level		10**	3**	2*	10**	8**	5**
Pooled correlation		0.668	0.426	0.214	0.537	0.299	0.314

B–C = body weight-cerebral weight; B–D = body weight-cerebral DNA; B–P = body weight-cerebral protein; C–D = cerebral weight-cerebral DNA; C–P = cerebral weight-cerebral protein; D–P = cerebral DNA–cerebral protein. * = Significant at 5-percent level; ** = significant at 1-percent level.

Table III. Correlation coefficients (r) for pooled neonatal parameters, and their significance (P). 249 neonatal rats, both sexes together

Parameters correlated	r	p
Body weight – cerebral weight	0.668	< 0.0005
Body weight – cerebral DNA	0.426	< 0.0005
Body weight – cerebral protein	0.214	< 0.0005
Cerebral weight – cerebral DNA	0.537	< 0.0005
Cerebral weight – cerebral protein	0.299	< 0.0005
Cerebral DNA – cerebral protein	0.314	< 0.0005
Cerebral weight/cerebral DNA – cerebral weight	0.572	< 0.0005
Cerebral protein/cerebral DNA – cerebral weight	0.691	< 0.0005
Cerebral weight/cerebral DNA – cerebral DNA	-0.380	< 0.0005
Cerebral protein/cerebral DNA – cerebral DNA	-0.124	$0.05 < p < 0.025$
Cerebral weight/cerebral DNA – cerebral protein	0.754	< 0.0005
Cerebral protein/cerebral DNA – cerebral protein	0.902	< 0.0005

cell. Similarly, the ratio cerebral protein/cerebral DNA is proportional to the amount of cerebral protein per one cerebral cell. However, these ratios obviously do not represent the *actual* volume or protein of one cell, because of the ventricles and the intercellular space [2].

As can be seen from table III in neonatal animals there is a significant negative correlation between the ratio cerebral weight/cerebral DNA and the cerebral DNA: thus, the more neonatal cerebral cells, the less space is allotted at this stage per each of them; this is despite the fact that the more cerebral cells (DNA) the higher total cerebral volume (weight). On the other hand, the negative correlation between cerebral protein/cerebral DNA and the cerebral DNA was not statistically significant.

The above considerations refer to normal (control) animals. The animals that suffer a decrease in neonatal cerebral cell number because of prenatal malnutrition [18, 21], or the animals that have an increase in this number because of special treatments [9, 10, 18] do not fall into this category. In such animals, the change in cerebral weight and protein is usually larger than the change in DNA, and therefore volume per cell and protein per cell may change in the same direction as DNA.

Other correlations (table III) reveal that the higher cerebral weight or total cerebral protein, the more space per cerebral cell or protein per cell, respectively, even though there are more cerebral cells.

4 of the 38 neonatal rats which had absolutely maximal body weight in their own litters were also maximal in the other three parameters, 7 were

Table IV. Signs of residuals for animals with maximal body weight

Regression on three remaining parameters				Regression on body weight alone		
	cerebral weight	cerebral DNA	cerebral protein	cerebral weight	cerebral DNA	cerebral protein
Regression within each litter						
	+20	19	18	16	20	15
	−18	19	20	22	18	23
Regression combined over all litters						
	+14	22	18	16	20	17
	−24	16	20	22	18	21

maximal in two of the other properties, and 12 were maximal in one. The remaining 15 rats which were maximal in body weight were maximal in none of the other measurements. To determine whether the tendency for the maximal rat to possess other maximal properties could be explained by the existence of correlations among the four parameters, two regressions were calculated for each of the brain parameters (weight, DNA, and protein). The first regressed each brain parameter on all three of the other parameters; the second regressed the brain parameter on body weight alone. Each regression was computed both using data only from the particular litter, and also using the pooled correlation estimates. If there were a tendency, other than that indicated by the existence of the positive correlations among the parameters, for the maximal rate to show extreme values of the other parameters, then more of the residuals (observed minus fitted values) for those rats should be positive than negative. Table IV shows the number of positive and negative residuals for each stiuation. In no case is there significantly more than 50% of the residuals which are positive. We may thus conclude that the numbers of maximal properties possessed by the largest rats in the litters are not inconsistent with the assumption that they arise from the correlation structure alone. A second approach to this analysis, not reported in detail here, involved simulation of litters of rats with the estimated correlation structure among the four parameters. The simulated results showed similar coincidences among maximal properties, further

Table V. Correlation coefficients (r) for pooled adult parameters, and their significance – 15 males and 92 females, pooled

Parameters correlated	Males		Females	
	r	p	r	p
Body weight–cerebral weight	0.548	$0.05 < p < 0.02$	0.051	> 0.1
Body weight–cerebral DNA	0.101	> 0.1	–0.113	> 0.1
Body weight–cerebral protein	0.678	$0.01 < p < 0.001$	–0.136	> 0.1
Cerebral weight–cerebral DNA	0.388	> 0.1	0.520	< 0.0005
Cerebral weight–cerebral protein	0.823	< 0.001	0.557	< 0.0005
Cerebral DNA–cerebral protein	0.238	> 0.1	0.512	< 0.0005
Cerebral weight/cerebral DNA–cerebral weight	0.740	< 0.005	–0.035	> 0.1
Cerebral protein/cerebral DNA–cerebral weight	0.641	< 0.01	0.014	> 0.1
Cerebral weight/cerebral DNA–cerebral DNA	–0.331	> 0.1	–0.868	< 0.0005
Cerebral protein/cerebral DNA–cerebral DNA	–0.250	> 0.1	–0.379	< 0.0005
Cerebral weight/cerebral DNA–cerebral protein	0.664	< 0.005	–0.607	< 0.0005
Cerebral protein/cerebral DNA–cerebral protein	0.880	< 0.0005	0.290	< 0.005

substantiating the position that the numbers of maximal properties are explainable by the correlations alone.

With respect to the adult animals one can see (table V) that the significance of correlations with body weight does not exist any more, presumably because of the multitude of unrelated postnatal factors that can affect body weight on one side, and brain parameters on the other side. The disappearance of significance of correlations (cortical dimensions) with age was also observed in our previous studies [3, 4]. Nevertheless, even in adult animals, if the number of animals is sufficiently large (females in this case) significant correlations still can be demonstrated for each pair of cerebral parameters: cerebral weight, DNA and protein. One must, however, consider that the meaning of these parameters at maturity might be different than at birth: cerebral weight and cerebral protein represent also noncellular substances, whereas cerebral DNA represent also postnatally arisen non-neuronal cells [1, 12, 17, 19], as well as polyploidy of some neurons [6]. One must also bear in mind that this overall statistical analysis does not

differentiate between diverse types of cells, within neuron and glia categories. Within these limitations, one can further see from table V that also for the adult animals the ratio cerebral weight/cerebral DNA shows a highly significant negative correlation with the cerebral DNA (total cell number) provided that the sample is large enough (here females). Thus, even in adult animals, the more cerebral cells, the less space is allotted to each of them. The negative correlation between the ratio cerebral protein/cerebral DNA (indicative of the cerebral protein content per one cerebral cell) and the cerebral DNA (total cell number) was also significant for the large sample (females); thus, the more cerebral cells the less protein there is per each.

Other correlations (table V) reveal that also in the adult animal the more total cerebral protein (but *not* the higher cerebral weight), the more space per cerebral cell and the more protein per cell, even though there are more cerebral cells.

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