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Correlations between Neonatal Body Weight and Adolescent Brain Development in Rats

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Abstract. Correlations between *neonatal* body weight, and body weight and several brain parameters *at 30 days* of age were studied in normal rats. At 30 days ('adolescence') cortex has already reached its final thickness and the rat exhibits long-term memory. Brain parameters included cerebral weight, DNA, protein and cholesterol contents and densities, as well as cortical and cerebral dimensions (cerebral sections). As expected, most of these parameters in 30-day-old animals were significantly correlated with each other. Unexpectedly, neonatal body weight was also significantly correlated with cholesterol content and density at 30 days, as well as with cortical and cerebral dimensions at 30 days.

Thus, statistically, neonatal body weight already predetermines the extent of neuronal (cerebral) development at adolescence (30 days). This finding also makes it possible to make at birth statistical predictions about future brain development without having to sacrifice neonatal animals.

Introduction

In previous papers we have reported the studies of correlations between body weights and some brain parameters in neonatal and mature rats (3, 4, 10). It was found that many parameters that were well correlated at birth, ceased to be correlated at adulthood. We have also studied neonatal rats with outstanding brain and body parameters (11).

In the present paper, we have extended these studies to the age (30 days, 'adolescence'), at which the cortex has already reached its final thickness (1), and yet this age is sufficiently close to birth to reveal correlations that do not exist anymore in mature animals. An attempt was also made to correlate parameters at 30 days with neonatal body weights, to predict future brain development without sacrificing the animals at birth. At 30 days, the animals already exhibit long-term memory (2) and can be subjected to adult behavioral tests (to be reported at a later date). The myelination is well advanced and cholesterol content can be

Table I. Mean values ± SD of parity[1], maternal weight[2], neonatal body weight, and 19 parameters for 87 30-day-old male rats

	Offspring									
	body at				cerebrum at 30 days				cortex at 30 days	
	0 days		30 days		right hemisphere		total			
Cerebral content										
Wet weight, g	6.95 ± 0.70	(C)	75.04 ± 14.75	(D)	0.518 ± 0.028	(E)	1.039 ± 0.054	(F)		
DNA, μg					503.07 ± 29.72	(G)	1002.35 ± 36.51	(H)		
Ratio H:F, μg/g							961.66 ± 37.45	(I)		
Protein, mg					37.56 ± 2.61	(J)	75.63 ± 5.37	(K)		
Ratio K:M, mg/mg							74.60 ± 5.04	(L)		
Cholesterol, mg					5.616 ± 0.536	(M)	11.09 ± 0.971	(N)		
Ratio N:F, mg/mg							10.73 ± 0.90	(O)		
Ratio N:H, mg/mg							11.16 ± 0.932	(P)		
Histological sections										
Width, mm^2									5.965 ± 0.261	(Q)
Thickness, mm									1.853 ± 0.083	(R)
Area, mm							39.36 ± 3.167	(S)	16.90 ± 1.257	(T)
Ratio, T:S									0.429 ± 0.0215	(U)

Letters in parentheses refer to ratios listed in the first column and to correlations listed in table II. Biochemical data determined for right hemisphere and extrapolated per total cerebrum. Dimensions for left hemisphere only. See text for details.

[1] Mean parity 1.82 ± 0.64 (A). First mating at 90 days.

[2] Mother's body weight (268.00 ± 35.88 g; B) determined at conception.

studied as an additional brain parameter. In addition, we studied cortical and total cerebral dimensions in 30-day-old animals and their correlations with other parameters.

Materials and Methods

The animals and their nutrition regimes were as described in the previous work (10–13). The rats were Sprague-Dawley-derived. Virgin females 3 months old and weighing 200–260 g were mated; the presence of a vaginal plug was considered day 0 of pregnancy and weight of the animals at that time was recorded. The animals were fed a pelleted stock diet containing 20.5% protein (Wayne Mousebreeder Block, Allied Mills, Chicago, Ill.). After normal delivery ($21^1/_2$ days of pregnancy), the male newborns were weighed, marked for identification, nursed by their mothers (and also had access to pelleted food) until weaning which in our laboratory is at the age of 30 days. They were then weighed, sacrificed, their cerebra (without olfactory lobes) dissected out and weighed, separated into two hemispheres and each weighed again; the left one was fixed in 10% buffered formalin for a minimum of 10 days (for histological study), and the right one stored at −15 °C for subsequent biochemical determinations.

Histological Study

Serial 20-μm frozen sections were cut, mounted and stained with cresyl echt violet for quantitative study. Stained sections at the rostral poles of the corpus callosum were selected and their projections (× 22.4) traced for subsequent measurements of cortex width, thickness and area as described previously (3, 4). In addition, total areas of the sections were also measured.

Biochemical Determinations

DNA was determined by a modification of the diphenylamine colorimetric method (8, 9). After the extraction of DNA, the pellets were resuspended in 10 ml of chloroform:methanol (2:1) mixture and left overnight so as to completely dissolve tissue-bound cholesterol. After centrifugation, cholesterol in the supernatant was determined by the colorimetric method of *Searcy and Bergquist* (7).

The pellets remaining after the extraction of cholesterol were used for the determination of protein by a modification of the colorimetric method of *Lowry et al.* (6).

Results and Discussion

Table I lists the mean values ± standard deviations for all parameters studied (pooled samples of 24 litters). In addition, we have calculated the following ratios: DNA:cerebral weight, which is an index of cerebral cell density; protein:DNA which is an index of cell size; cholesterol:cerebral weight, and cholesterol:DNA, which are indices of myelin density and of myelin content per cell, respectively. It is assumed here that the specific gravity of the cerebrum is essentially constant so that cerebral weight is proportional to cerebral volume. In histological studies, besides dimensions and areas, we have also determined the ratio cortex area:total cerebral area of the section, which is an index of the *relative* cortical volume. It is to be noted that while individual areas on the slide show 7.4–8% variability, due mostly to non-identical shrinkage during the fixation, the *ratios* of areas as represented here show only 5% variability (calculated as standard deviation, in percent of the mean).

The correlations between all parameters (5) are presented in table II. It can be seen that many parameters show statistically significant correlations with each other. Parity (A) is significantly positively correlated with maternal weight at conception (B), but negatively with offspring weight at birth (C) and at 30 days (D). This suggests that in the rat the *first* pregnancy is more advantageous for the size of the offspring. In this context, the negative correlation of maternal body weight (B) with offspring weight at birth (C) and at 30 days

(D), as well as borderline correlation with total DNA (H) and cortical area (T) may merely mean that second and third pregnancy (higher maternal body weight) is also less advantageous for body and brain development of the offspring; a separate study *within* first pregnancy (not in the table) reveals significant *positive* correlation between maternal body weight and offspring neonatal body weight ($r = 0.77$; $p < 0.05\%$) and brain weight ($r = 0.86$; $p < 0.01\%$).

Offspring weight at birth (C) shows only borderline correlation with offspring weight at 30 days (D), undoubtedly due to unequal postnatal nutrition (during nursing). However, unexpectedly, offspring weight at birth (C) showed highly significant correlations with cholesterol content (M, N), cholesterol:cerebral weight (O; index of cerebral cholesterol density) and cholesterol:DNA (P; index of cholesterol content per cerebral cell), as well as cortical section area (S), and borderline correlations with cortical width (Q) and thickness (R). The correlations of birth weight (C) with total cerebral DNA content (H) and total cerebral protein content (K) are positive but do not reach the level of significance; possibly, they would be significant if the sizes of nursing litters were standardized, or if the total number of animals were higher.

Table II. Correlation coefficients between parameters in table I, and their significance (87 male rats)

Parameters	B	C	D	E	F	G	H	I	J	K
A	0.51[3]	−0.46[2]	−0.39[2]	−0.07	−0.12	−0.05	−0.09	0.05	−0.01	0.01
B	1	−0.56[3]	−0.34[2]	−0.09	−0.12	0.04	−0.27[1]	0.16	−0.13	−0.01
C		1	0.40[1]	0.21	0.28	0.19	0.31	0.00	0.25	0.33
D			1	0.51[3]	0.63[3]	0.09	0.26[1]	−0.36[2]	0.40[3]	0.32[1]
E				1	0.89[3]	0.74[3]	0.68[3]	−0.07	0.67[3]	0.67[3]
F					1	0.63[3]	0.75[3]	−0.44[3]	0.69[3]	0.70[3]
G						1	0.87[3]	0.62[3]	0.38[2]	0.53[3]
H							1	0.26[1]	0.57[3]	0.56[3]
I								1	−0.18	−0.26[1]
J									1	0.96[3]
K										1
L										
M										
N										
O										
P										
Q										
R										
S										
T										

Parameter symbols as in table I.

[1] Significant at $<5\%$ level (two-tailed test).

[2] Significant at $<1\%$ level.

[3] Significant at $<0.1\%$ level.

Cholesterol content increases with the thickness of myelin and with the degree of dendritic arborization, and therefore cholesterol content may be considered an index of neuronal development (deployment). Thus, all the above correlations (M, N, O, P, Q, R, T) with birth weight (C) suggest that in rats the neonatal body weight, known to be correlated with neonatal brain parameters (10), already predetermines the extent of neuronal (cerebral) development, at least at adolescence (30 days). This finding also makes it possible to make at birth statistical predictions about future brain development, without having to sacrifice neonatal animals.

Other correlations are less unexpected. Body weight at 30 days (D) is well correlated with practically all brain parameters studied. This follows the correlations found for neonatal and 10-day-old rats (3, 10); however, many correlations which would have already diasappeared in adult rats (3, 10), are still visible in adolescent rats (30 days). Thus, cerebral DNA content (H) at 30 days is well correlated with protein (J, K) and cholesterol (M, N) contents as well as with cerebral section area (S). As expected, DNA: cerebral weight (I; index of cell density) is *negatively* correlated with cerebral weight (F) and with protein:DNA (L; index of cell size), and the latter (L) is negatively correlated with

	L	M	N	O	P	Q	R	S	T	U
A	0.02	−0.10	−0.13	−0.09	−0.10	−0.24	−0.12	−0.27	−0.29	0.03
B	−0.17	−0.03	−0.32[1]	0.01	−0.06	−0.32	−0.08	−0.25	−0.37[1]	0.13
C	0.11	0.53[2]	0.59[3]	0.52[2]	0.54[2]	0.40[1]	0.40[1]	0.51[2]	0.34	0.12
D	0.36[2]	0.36[2]	0.58[3]	0.19	0.37[2]	0.42[2]	0.37[1]	0.59[3]	0.64[3]	−0.10
E	0.08	0.50[3]	0.39[2]	0.01	0.04	0.08	0.18	0.37[1]	0.38[1]	−0.07
F	0.39[2]	0.47[3]	0.48[3]	0.02	0.16	0.16	0.38[1]	0.56[3]	0.55[3]	−0.05
G	−0.41[3]	0.55[3]	0.27[1]	0.20	−0.09	0.20	0.29	0.52[2]	0.25	0.21
H	0.05	0.36[2]	0.36[2]	0.02	−0.08	0.25	0.34	0.51[2]	0.36	0.08
I	−0.67[3]	0.22	−0.21	0.28[1]	−0.19	−0.07	−0.18	−0.15	−0.21	0.09
J	0.69[3]	0.24[1]	0.30[1]	−0.08	0.00	0.14	0.48[1]	0.64[3]	0.22	0.34
K	0.82[3]	0.26[1]	0.23	−0.11	−0.02	0.19	0.56[2]	0.65[3]	0.30	0.27
L	1	−0.21	0.18	−0.29[1]	0.04	−0.01	0.43[1]	0.38[1]	0.06	0.26
M		1	0.97[3]	0.87[3]	0.78[3]	0.28	0.46[1]	0.60[3]	0.44[1]	0.06
N			1	0.89[3]	0.90[3]	0.31	0.49[2]	0.57[2]	0.48[1]	−0.01
O				1	0.89[3]	0.22	0.35	0.38[1]	0.30	−0.01
P					1	0.21	0.35	0.34	0.31	−0.05
Q						1	0.28	0.64[3]	0.73[3]	−0.19
R							1	0.73[3]	0.48[2]	0.33[1]
S								1	0.80[3]	0.22
T									1	−0.41[2]

cholesterol:cerebral weight (O), because the higher the cell density, the smaller the cell and the higher cholesterol density. Cholesterol content is well correlated with cortical and total cerebral dimensions. The remaining correlations are also essentially as expected.

In conclusion, adolescent (30 days) rats exhibit significant correlations between their brain and body parameters, just as did neonatal animals (3, 10). An unexpected finding was that *neonatal* body weight is significantly correlated with cerebral development (cholesterol, cortical and other cerebral dimensions) at 30 days, i.e. at the age at which the cortex thickness is essentially final (1) and the animal already exhibits long-term memory. Thus, it is possible to predict, from neonatal body weight, the concomitant brain development at *30 days;* such estimate would be statistically valid for a population, but considerably less valid for an individual.

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