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NEONATAL RATS WITH OUTSTANDING VALUES OF BRAIN AND BODY PARAMETERS

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"Outstanding" newborn rats, with parameter values more than two standard deviations above the mean (OH) or below the mean (OL), were identified in a normal population of 720 animals and were studied for correlations between these parameters: body weight, brain weight, brain DNA (cell number) and brain protein. The animals OH on any one of these parameters were also higher than average on all the others, and some of these animals came from OH litters. The animals OL on one parameter tended to be OL on all the others, and correlations between parameters were higher in this group than in the entire population. Such findings may facilitate the search for OH or OL individuals. The correlations among the OH parameters were not the same as the correlations among the OL. The causes of the occurrence of OH and OL animals are more likely to be environmental than genetic.

In a previous paper (1) we have established correlations between body weights and brain parameters in a large sample of newborn and mature rats. In the present work we focus our attention on rats with outstanding high (OH) or outstanding low (OL) values of brain and body parameters spontaneously occurring in such large populations. We define as "outstanding" an individual in which the value of any parameter under study is more than two standard deviations above (OH) or below (OL) the mean value for the full population; the term is not meant to prejudge that this must be of advantage or of disadvantage to the individual. Similarly, an "outstanding litter" is defined as the one whose mean value of a parameter is more than two standard errors above (OH) or below (OL) the mean value for the population. In this paper such OH and OL animals were identified among 720 newborns and were studied for correlations between these parameters: body weight, brain weight, brain DNA (cell number) and brain protein. The study was limited to newborn animals, in view of our previous findings (1-3) that in mature animals the correlations between body weights and brain parameters become less distinct. The animal outstanding at birth may not remain outstanding when it reaches adulthood; this subject is under study and will be reported at a later date.

Materials and Methods

We used albino rats derived from the Sprague-Dawley strain, bred in our laboratory as a closed colony. The animals in this study were from the 33rd to the 37th generation; the dams were primiparous, 3 month old, and weighed 200 to 260 g. The animals were maintained on pelleted diet (Wayne Mousebreeder Block) ad lib. (average intake 16 g/day).

The 720 newborns (81 litters; years 1973-74) were weighed, and then killed by decapitation, within 6 hours of delivery. The brains (cerebral hemispheres only) were immediately removed without cerebellum and olfactory lobes and weighed; they were then frozen and subsequently used for DNA (4, 5) and protein (6) determination as described previously (1).

Results and Discussion

The identification of individual newborn OH and OL rats as well as OH and OL litters is represented in Table I. The fractions of OH and OL animals vary from approximately 1 to 4 percent of the total, depending on the parameter. The distribution of the four parameters is essentially Normal, except for body weights which exhibit slight negative skewness (2.28% of a Normal population lies more than two standard deviations above or below the mean).

Table II shows that for the entire population all the parameters are highly correlated (1) ($p<0.0001$). Correlations in the tails of a truncated multivariate Normal population are generally smaller than those in the entire population. This feature affects all variables, not only those which are the subject of truncation. Since selection of OL and OH animals with reference to a particular parameter amounts to truncation of that parameter, we should expect the correlations computed from the OL and OH animals to resemble correlations theoretically derived from truncating a multivariate Normal population. Table II shows theoretical values r_o for the correlations among the four parameters based on the correlations in our full population of 720 rats and on truncation at $\pm$ 2 standard deviations on each of the parameters. These values were computed by numerical integration from the appropriate trivariate Normal probability density function. We know of no statistical test to compare the observed correlations r_1 in OL and OH animals with those based on theoretical considerations. We note, however, that the observed correlations for OH animals are generally similar to their theoretical counterparts, whereas the observed correlations for OL animals tend to be much larger than those computed from the truncated Normal populations. Thus, the OH animals appear to merely come from the upper tails of a Normal population, and the tendency for an animal which is OH on one parameter to be OH on another is no greater than would be expected from the population correlations. This is in contrast to the OL animals: an animal OL on one parameter tended to be OL on all the others and correlations between parameters were higher in the OL group than in the entire population. Thus, the correlations among the OH parameters are not the same as the correlations among the OL. These results may be somewhat analogous to the situation in human newborns (7) where "maternal constraint in women is pre-potent at the lower extreme (of birth weight); at the upper extreme, relaxation of constraint permits other factors to take up more of the variance".

Table III shows that even with the small population of OH animals, an animal OH on one parameter tends to have values of other parameters higher than the mean for the population. Conversely, an animal OL on one parameter tends to have values of other parameters lower than the mean for the population.

Table IV shows that some of the OH animals and most of the OL animals came from litters whose mean value of the parameter on which they were outstanding was also OH or OL, respectively ("OH litters or OL litters", see introduction); the percentages of such animals are significantly higher ($p< 0.001$) than would be expected if there were no correlations among the parameters. Especially among litters designated OL on one of the parameters, the occurrence of OL individuals on that parameter and on other parameters is high.

The results shown in Tables II and IV may facilitate the search for such OH and OL individuals. For example, on a statistical basis, an individual

TABLE I

Identification of OH and OL individual newborns*, and OH and OL litters** (rats)

		Entire population				OH with respect to				OL with respect to			
		Body weight	Cerebral weight	Cerebral DNA	Cerebral protein	Body weight	Cerebral weight	Cerebral DNA	Cerebral protein	Body weight	Cerebral weight	Cerebral DNA	Cerebral protein
Individual animals	Number	720	720	720	720	7	10	16	23	27	23	16	15
	Percent of total	100	100	100	100	0.97	1.39	2.22	3.19	3.75	3.19	2.22	2.08
	Mean value (mg) ± std. dev.	5978 ±715	161.5 ±17.5	0.5597 ±0.0344	8.75 ±1.27	7900 ±428	203.4 ±5.78	0.6464 ±0.0200	11.74 ±0.40	4019 ±471	115.5 ±7.25	0.4765 ±0.0147	5.77 ±0.31
Litters	Number	81	81	81	81	0	2	1	3	5	3	3	4
	Percent of total	100	100	100	100	0	2.5	1.2	3.7	6.2	3.7	3.7	4.9

Parameter values more than 2 standard deviations (*) or more than 2 standard errors (**) above the mean for the entire population. Standard errors were determined by using components of variance estimated from litter-to-litter and within-litter variation. Both sexes together. Cerebrum without olfactory lobes.

TABLE II

Correlations coefficients for the entire population and for the OH and OL animals

	Entire population		OH or OL with respect to											
			Body weight			Cerebral weight			Cerebral DNA			Cerebral protein		
Parameters correlated	r	p	r_o	r_1 OH	r_1 OL	r_o	r_1 OH	r_1 OL	r_o	r_1 OH	r_1 OL	r_o	r_1 OH	r_1 OL
n	720		16.4	7	27	16.4	10	23	16.4	16	16	16.4	23	15
Body weight-cerebral weight	0.758	<0.0001	0.350	0.356	0.712	0.350	0.085	0.744	0.713	0.721	0.845	0.655	0.446	0.920
Body weight-cerebral DNA	0.414	<0.0001	0.147	-0.254	0.551	0.211	-0.414	0.586	0.148	0.041	0.280	0.262	0.387	0.716
Body weight-cerebral protein	0.535	<0.0001	0.199	0.127	0.579	0.172	-0.392	0.723	0.445	0.365	0.721	0.199	0.067	0.574
Cerebral weight-cerebral DNA	0.410	<0.0001	0.201	0.041	0.460	0.145	-0.196	0.703	0.146	0.490	0.300	0.221	0.370	0.879
Cerebral weight-cerebral protein	0.648	<0.0001	0.472	-0.195	0.814	0.264	0.527	0.562	0.580	0.348	0.948	0.264	0.138	0.608
Cerebral DNA-cerebral protein	0.431	<0.0001	0.292	0.016	0.580	0.264	0.107	0.713	0.156	-0.283	0.222	0.155	-0.065	0.663

r = Correlations for the entire population
p = Probabilities for the entire population
r_o = Correlation which would be expected in truncated population, computed by numerical integration of a truncated trivariate Normal distribution
r_1 = Correlation observed in truncated sample
See Table I for other explanations

TABLE III

Percentages of individuals OH or OL at birth with respect to one parameter who also have other parameters higher or lower than the mean for the entire population

		Individuals OH with respect to				Individuals OL with respect to			
		Body weight	Cerebral weight	Cerebral DNA	Cerebral protein	Body weight	Cerebral weight	Cerebral DNA	Cerebral protein
Other parameters	Body weight	100	100***	75*	86.96***	100	100***	62.5	93.3***
	Cerebral weight	100**	100	68.75	91.30***	100***	100	81.25*	93.3***
	Cerebral DNA	100**	90**	100	73.91**	96.3***	95.7***	100	93.3***
	Cerebral protein	100**	90**	75*	100	92.6***	100***	81.25*	100

* Significantly greater than the expected 50%, at the 0.05 level.
** Significantly greater than the expected 50%, at the 0.01 level.
*** Significantly greater than the expected 50%, at the 0.001 level.
See Table I for other explanations

TABLE IV

Percentages of individuals outstanding at birth who came from outstanding litters

		Individuals OH with respect to				Individuals OL with respect to			
		Body weight	Cerebral weight	Cerebral DNA	Cerebral protein	Body weight	Cerebral weight	Cerebral DNA	Cerebral protein
Litters OH or OL with respect to	Body weight	0	0	0	0	96.3**	82.61**	56.25**	80**
	Cerebral weight	0	50**	6.25**	4.35	92.53**	100**	62.5**	80**
	Cerebral DNA	0	0	31.25**	0	77.78**	65.22**	68.75**	86.67**
	Cerebral protein	0	20**	0	43.48**	88.89**	95.65**	62.5**	93.33**

* Significantly greater than the expected 2.28%, at the 0.05 level.
** Significantly greater than the expected 2.28%, at the 0.001 level.
See Table I for other explanations

which at birth had OH cerebral cell number (DNA), is likely to be the one which also had higher than average birth weight, and the one which came from the litter OH with respect to cerebral cell number. Conversely, an individual which at birth had OL cerebral cell number (DNA), is likely to be the one which also had lower than average birth weight, and the one which came from the litter OL with respect to cerebral cell number.

In the newborn rat, the cerebral cells are mostly neurons, and their number at birth is final or nearly final (review in (8)); thus, an OH animal may well retain the superiority in neuron number when adult. Other parameters, such as cerebral protein content, may not remain OH in the adult, but nevertheless such superiority at birth may exert beneficial influence during postnatal neuron differentiation. The converse may be true for OL animals: inferiority at birth may exert harmful influence during postnatal neuron differentiation.

The occurrence of newborns with OH or OL amounts of cerebral DNA (cell number) indicates that the known remarkable constancy of this parameter at birth can be circumvented by natural causes. The occurrence of OH animals has been reported rarely (8) and the causes are essentially unknown.

The gestation period of OH animals (21 to 22 days, counting the presence of a vaginal plug as day 0) was the same as for the entire population; this suggests that OH are not just "older" average animals. In addition, DNA values around term reach a plateau (8) so that an older animal would not be OH with respect to this parameter. Similarly, the OL are not just "younger" average animals. However, we are conducting further studies to exclude entirely the possibility of some cryptic age difference.

The occurrence of OH and OL animals is also not apparently due to any systematic difference in number in the litter (litter size): the mean litter size (weighted average) was 10.09 for the entire population, and between 9.38 and 10.81 for animals OH or OL with respect to body weight, cerebral DNA, and cerebral protein; these differences were not statistically significant. For animals OH with respect to cerebral weight the average litter size was 7.50 and for animals OL with respect to cerebral weight it was 8.43. Both of these values are significantly less than the mean litter size for animals which are neither OH or OL, hence there does not appear to be a causal relationship. Furthermore, for all four parameters there was greater variability among litters of equal sizes than among different litter sizes.

It might be hypothesized that the occurrence of OH or OL animals is due to their special genotype. While at present this cannot be totally excluded, it must be pointed out that the rats used in the present study were bred by us as a closed colony for 33 to 37 generations; thus, the genetic variability is less likely to be the explanation of the occurrence of OH or OL animals. An indirect indication has also been obtained in the studies of mouse lines. Lines of mice with higher (line H) or lower (line L) adult brain weight have been selected by others (9-11) and found by us to have significantly higher (line H) or lower (line L) body weight, cerebral weight, cerebral DNA and cerebral protein contents also at birth (12). However, the means for these parameters in each line were at birth less than 2 std. deviations above (line H) or below (line L) the means for unselected control, i.e., each line per se was not OH or OL at birth in terms of the present paper. We have also found that the percentage of newborn H individuals that were OH with respect to the mean of H line (4.2% for cerebral weight) was not much lower than the percentage of newborn unselected controls that were OH with respect to the mean of controls (4.8%); this indicates that the past genetic stabilization of such selected H line (18 generations of selective breeding) did not abolish the occurrence of OH animals in this line. Thus, such occurrence is less likely to be caused by genetic

factors.

It has often been maintained that for many species, including human, hereditary correlations as such can largely be disregarded for determination of the size of the newborn, the correlations here being mainly environmental (review in (13)); however, prenatal brain development has not been studied in this respect. Our finding that the newborns OH with respect to brain parameters also have body weights higher than the mean for the population, suggests that for the neonatal brain, too, the causes may be environmental, such as the general optimal intrauterine conditions, including optimal prenatal nutrition. Such conditions might have prevailed for each OH litter, but might have been especially favorable for the OH fetuses within such litters. The improvement of newborn brain parameters by experimental improvements of intrauterine conditions has indeed been demonstrated (14-17).

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