

## The Quantitative Relationship between Nutritional Effects on Prewaning Growth and Behavioral Development in Mice

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### **Abstract:**

Our objective was to establish whether nutritional effects on the behavioral development of preweaning mouse pups were linearly related to effects on body and brain growth or whether there was a threshold effect, with behavior being affected only by nutritional extremes. We also used a standardized scale of development to compare the relative magnitude of such effects on morphological and behavioral measures. The level of nutrient availability was manipulated continuously by rearing the pups in litter sizes ranging from 3 to 12. On Day 32 post-conception, measures were taken of body weight, brain weight, thickness of the cerebellar external granular layer (EGL), and behavioral development. The relationship between litter size and body weight, brain weight, and behavioral development was best described by a linear regression model; no threshold effect was apparent. By comparing measures on animals from different litter sizes at the same age (32 days) to standard developmental curves over a wide range of ages, we found that for every additional pup in a litter, body growth was retarded by the equivalent of 1.28 days, brain weight by 0.44 day, and behavioral development by 0.07 day. Although the variation in nutrient availability provided by this range of litter sizes does result in a linear relationship between growth and behavioral development, there is nevertheless considerable sparing of function.

### **Article:**

#### ***Introduction***

An important question related to the effects of malnutrition on behavioral development is that of the nature of the relationship between growth decrement and functional impairment, (Margen, 1984). There appear to be two schools of thought on this: those who argue that any decrease in size will be associated with functional impairment and those who hold that the functional integrity of the organism will be preserved within certain homeostatic bounds and that only when these are exceeded will functional impairment result. Continuous functional effects would be supported by a linear relationship between growth and function, whereas a curvilinear relationship would be more supportive of a threshold effect. A less common question, but one which is relevant in this context, is whether overnutrition is associated with an acceleration of development. The type of quantitative analysis necessary to answer questions of this type demands a continuous range of nutrient availability during development. Therefore, in the present study, preweaning nutrition was manipulated by rearing mice over a range of litter sizes in order to investigate the nature and magnitude of such effects on body and brain growth and behavioral development.

The effects of malnutrition on brain and behavioral development have been reviewed extensively (Bedi, 1984; Dobbing and Smart, 1973; Leathwood, 1978; Morgane et al., 1978; Resnick & Morgane, 1983; Smart, 1977, 1986; Zamenhof & Van Marthens, 1978). It has been suggested that a developing organ or system is most susceptible to insult during the time of most rapid growth. In rodents the time during which the brain shows its greatest rate of increase and during which malnutrition leads to permanent decreases in brain weight is the preweaning period. However, because of the heterogeneity of brain tissue, the timing of this vulnerable period for individual systems within the brain may vary. In general, the macroneuronal populations are formed

prenatally, whereas postnatal growth is a reflection of microneuronal and glial proliferation, as well as axonal and dendritic outgrowth and the formation of synapses. This is well illustrated in the cerebellum, where Purkinje cells originate prenatally whereas the granule cells are postnatal in origin. This has led to the suggestion that the cerebellum may be a useful model for investigating nutritional effects at different time periods during development (Bedi, 1984). Despite the evidence that the growth of the brain is susceptible to undernutrition during the preweaning period, a consistent finding is that of "brain sparing" where, proportional to the effect on the body, that on the brain is very much smaller. For example, a recent study showed that, after nutritional rehabilitation, previously undernourished rats showed a body weight deficit of 23%, whereas the magnitude of the deficit in the forebrain and cerebellum were 7 and 11%, respectively (Warren & Bedi, 1985). Nevertheless, nutritional insult during the period of brain development results in brains which are smaller and structurally distorted, as well as effects on brain chemistry and behavior.

Much of the previous work which has manipulated preweaning nutrition in rodents by varying litter size has done so categorically by using extreme litter sizes, with over- and undernutrition represented by small and large litters respectively; some studies have included a medium sized litter as a control group. With respect to body weight, pups raised in small litters may show enhanced growth relative to controls, whereas those raised in large litters are generally stunted (Altman et al., 1971; Aubert, 1980; Castellano & Oliverio, 1976; Hausberger & Volz, 1984; Milkovic, Paunovic & Joffe, 1976; Jen, Wehmer & Morofski, 1978; Wehmer & Jen, 1978). Two studies which included a range of litter sizes varied in outcome; mice showed an inverse relationship between litter size and weaning weight (except for litters of 2) (Epstein, 1978), whereas rats showed an effect of large litters only (Wurtman & Miller, 1976). Epstein interpreted the retarded growth of mice in litters of 2 as being attributable to insufficient stimulation of the mother by the pups and therefore inadequate lactation.

Although brain growth is also affected by postnatal litter size, there is sometimes indication of brain sparing in pups from large litters (Altman et al., 1971; Castellano & Oliverio, 1976; Wehmer & Jen, 1978). There is also evidence that the development of the cerebellum, which is occurring rapidly during this time period, is affected more severely than the cerebrum (Chase, Lindsley & O'Brien, 1969; Neville & Chase, 1971; Wehmer & Jen, 1978). Behavioral studies show that large litter rearing does retard behavioral development (Castellano & Oliverio, 1976; Wehmer & Jen, 1978), but the nature and magnitude of such effects relative to those on morphological development have not been assessed.

Comparisons based on a correlational approach merely indicate the relative ordering of subjects on two variables, and do not necessarily imply a similar magnitude of effects on each variable. For example, increasing litter size may retard both body (or brain) growth and behavioral development such that there is a strong linear relationship between growth decrement and function. Nevertheless, even in the absence of a threshold effect, the magnitude of the effects on behavior may be reduced relative to those on growth, implying some sparing of function. One way of assessing this is to convert each variable to a common scale, thereby making direct comparison possible. The approach taken in this study was to use a standardized developmental scale to achieve this end.

Wahlsten (1974, 1975) developed this scale to describe the relationship between chronological age and developmental indices, including body weight, brain weight, thickness of the cerebellar external granular layer (EGL) and behavioral development in mice. Behavioral development was assessed using a battery of tests which measured both motor and sensory function. The population used was the F<sub>2</sub> offspring of B6D2F<sub>1</sub> hybrid mice, which are characterized by robustness and reproductive vigor. A standard series of separate litters of B6D2F<sub>2</sub> offspring, ranging in age from 27 to 36 days post-conception (about 8-17 days after birth), was tested on each of these variables. Then a quadratic regression equation relating chronological age to each variable was derived from litter mean scores. By substituting the actual score ( $x$ ) of a developing animal into this equation, one is able to obtain its developmental age ( $y$ ), which is the age at which the standard B6D2F<sub>2</sub> mice are expected to reach that value. The use of these equations in an experimental situation allows, for example, comparison of developmental age as derived from body weight with that derived from behavioral development. This procedure is superior to comparisons based on percentage differences in brain and body weight or

behavioral measures because differences in rate of development produced by differences in nutrition are judged with respect to the rate of normal growth for all measures, as indicated by a common scale of measurement, days of normal growth.

In the present study B6D2F<sub>2</sub> mice were reared in litters ranging in size from 2 to 12 pups and tested on the developmental scale on day 32 post-conception (about 13 days after birth). Pups from average-sized litters of 8 were similarly tested at each of days 31, 32, and 33 in order to demonstrate the sensitivity of the scale. Based on the findings described in the literature, increasing litter size would be expected to retard development. The magnitude of the retardation can then be determined empirically, using the methods outlined above.

## **Method**

### ***Subjects***

Parental B6D2F<sub>1</sub> hybrid mice, obtained from Charles River Breeding Laboratories, St. Constant, Quebec, were mated to obtain male and female B6D2F<sub>2</sub> offspring. Females were nulliparous and 12 to 16 weeks old at initial mating. They were housed in groups of 4 to 5 until they were pregnant, whereafter they were housed separately. On the day of parturition the females were assigned randomly to rearing litters ranging from 2 to 12 pups. Two litters of each size were formed, except for litters of 2, where 3 litters were formed. Animals had free access to lab chow (Maple Leaf Mills, Toronto) and tap water, and were housed in opaque plastic cages measuring 29 × 18 × 13 cm. They were maintained on a reversed 12-hr light/12-hr dark schedule, with the dark period between 0800 and 2000 hours. Beta-chip hardwood was provided for bedding and two sheets of toilet tissue for nesting material. Temperature was maintained at 22 ± 1°C.

### ***Procedure***

The animals were mated at the beginning of the dark cycle and the males removed 6 hr later. Females were checked for the presence of a vaginal plug and if detected, the female was housed separately and the day of conception recorded as day 0. Pregnant females were checked daily at 0830 hr for births. Pups born on the same day were pooled, sexed, and randomly reassigned to mothers, which had in turn been randomly assigned to litter sizes. An effort was made to assign equal numbers of male and female pups to each litter. Litters were left undisturbed until testing. Pups from different litter sizes were tested on day 32 post-conception, and 2 extra litters of 8 pups each were tested at each of days 31 and 33 to demonstrate the sensitivity of the developmental scale.

On the day of testing, mothers and pups were weighed and 2 pups of each sex, with weights closest to the litter mean for their sex, were chosen for behavioral testing (except for litter size less than 5, where all pups were tested). These pups were coded so that testing was done without prior knowledge of their age, litter size, or precise body weight. In addition, all pups to be tested on the same day were presented individually to the experimenter in a random order, so that not all pups from the same litter were tested sequentially. Pups were subjected to the following sequence of behavioral tests.

- (1) EYES OPEN. Are both eyes fully open?
- (2) RIGHTING REFLEX. Does subject return rapidly to its feet when placed on its back?
- (3) CLIFF AVERSION. Does subject withdraw from the edge of a flat surface when its snout and forepaws are placed over the precipice?
- (4) FORELIMB AND HINDLIMB GRASP REFLEX. Does subject grasp strongly the barrel of an 18-gauge needle when it is touched to the palm of each forepaw and hindpaw?
- (5) VIBRISSA PLACING REFLEX. Does subject place its forepaw onto a cotton swab which is stroked across its vibrissae?

- (6) LEVEL SCREEN TEST. Can subject hold onto a piece of 288-mesh aluminum screen when it is dragged across it horizontally by the tail?
- (7) VERTICAL SCREEN TEST. Can subject hold onto the screen when it is placed in a vertical position?
- (8) SCREEN CLIMBING TEST. Can subject climb up the vertical screen using both forelimbs and hindlimbs?
- (9) POLE GRASP. Can subject grasp the shaft (2.5 mm.) of a cotton swab with both forepaws and hindpaws?
- (10) FORELIMB AND HINDLIMB STICK GRASP. Can subject grip firmly a 9.5 mm-wide wooden stick with its forepaws and hindpaws?
- (11) VISUAL PLACING REFLEX. Does subject extend its forelimbs when it is lowered rapidly towards a flat surface?
- (12) AUDITORY STARTLE RESPONSE. Does subject show a whole-body startle response when a paper clip (snap type) is snapped less than 6 in. away?

Scores for each of the 12 tests (ranging from 0 to 1.0) were averaged to yield a mean behavioral score for each subject.

Immediately after testing, one animal of each sex was selected randomly from each litter and was anesthetized with an overdose of sodium pentobarbital, followed by intracardiac perfusion with 10% buffered formalin. The brain was removed, stored in 10% formalin, and weighed one week later. Prior to weighing, the brains were trimmed by removal of the paraflocculi and olfactory bulbs and by a perpendicular cut through the brain stem immediately caudal to the cerebellum. They were then embedded in 10% gelatin and stored in formalin until they were sectioned. Serial sagittal sections of 25  $\mu$ m were cut with a freezing microtome and stained with formolthionin for Nissl substance. The thickness of the cerebellar EGL was measured as described previously (Wahlsten, 1974, 1975) using the section closest to the midsagittal plane, and viewed at 1000  $\times$  magnification under oil immersion. Briefly, ten separate measurements were taken over the five major cerebellar fissures and averaged to yield one measurement for each brain. It should be noted that the EGL consists of a layer of cells on the surface of the cerebellum which, in rodents, increase in number during the first postnatal week, but which then cease proliferating and migrate inward to the molecular and internal granular layers (Jacobsen, 1978). Therefore the thickness of the EGL decreases, rather than increases, with increasing age during the period under study (Wahlsten, 1974). The equations used to predict age ( $y$ ) from each variable ( $x$ ) separately are as follows (Wahlsten, 1974,<sup>1</sup> 1975<sup>2</sup>)

$$^1\text{Body weight: } y = 24.35 - 0.35x + 0.21x^2$$

$$^1\text{Brain weight: } y = 51.76 - 188.36x + 356.19x^2$$

$$^1\text{EGL thickness: } y = 35.66 - 0.54x + 0.01x^2$$

$$^2\text{Behavioral score: } y = 24.40 + 11.14x - 1.09x^2$$

### *Analysis*

The data were analyzed using the general linear model (GLM) procedure provided by Statistical Analysis Systems (SAS) to do regression analysis on the relationship between litter size and pup development. The age differences between the standard litters as well as a post-hoc test of differences between individual litter sizes were analysed by a one-way analysis of variance combined with Tukey's test to compare individual group means. All analyses were computed using the litter mean score as the unit of analysis. The alpha level was set at  $p < 0.05$ .

## Results

Preliminary analysis of the data including sex as a variable indicated an effect of sex on body weight only ( $F(1,35) = 4.34, p < 0.05$ ), with males heavier than females. As there was no sex by litter size interaction, further analyses were done with the data collapsed across sex.

The data on each developmental measure as a function of litter size are shown in Table 1.

**TABLE 1. Development of B6D2F<sub>2</sub> Mice on Day 32 Post-Conception as a Function of Litter Size.**<sup>a,b,c</sup>

Litter Size	Body Weight (g)	Brain Weight (g)	EGL Thickness ( $\mu\text{m}$ )	Behavioral Score
2	7.7 $\pm$ 0.2	0.401 $\pm$ 0.009	12.5 $\pm$ 0.5	0.74 $\pm$ 0.006
3	9.6 $\pm$ 0.2	0.440 $\pm$ 0.004	13.3 $\pm$ 1.3	0.80 $\pm$ 0.02
4	9.4 $\pm$ 0.2	0.423 $\pm$ 0.006	14.0 $\pm$ 1.5	0.82 $\pm$ 0.004
5	9.2 $\pm$ 0.4	0.430 $\pm$ 0.004	11.0 $\pm$ 0.0	0.85 $\pm$ 0.02
6	8.4 $\pm$ 0.1	0.421 $\pm$ 0.017	12.3 $\pm$ 0.7	0.82 $\pm$ 0.02
7	7.9 $\pm$ 0.5	0.419 $\pm$ 0.009	12.5 $\pm$ 0.5	0.79 $\pm$ 0.02
8	7.1 $\pm$ 0.1	0.411 $\pm$ 0.003	14.5 $\pm$ 0.5	0.79 $\pm$ 0.02
9	6.9 $\pm$ 0.3	0.410 $\pm$ 0.023	12.8 $\pm$ 2.3	0.76 $\pm$ 0.02
10	6.5 $\pm$ 0.2	0.408 $\pm$ 0.014	12.8 $\pm$ 0.3	0.75 $\pm$ 0.003
11	6.7 $\pm$ 0.5	0.421 $\pm$ 0.014	11.5 $\pm$ 1.0	0.80 $\pm$ 0.02
12	5.8 $\pm$ 0.3	0.384 $\pm$ 0.017	13.3 $\pm$ 0.7	0.77 $\pm$ 0.02

<sup>a</sup> Data are presented as means  $\pm$  SEM.

<sup>b</sup> Data were analyzed by collapsing across sex and using the litter mean score as the unit of analysis.

<sup>c</sup>  $n = 2$  litters per litter size, except for 3 litters of litter size = 2.

As predicted by the literature, pups raised in litters of 2 did not appear to lie on the same continuum as those from other litter sizes. This was confirmed by the regression analysis, where inclusion of litters of 2 resulted in a model which incorporated a quadratic component and lacked the predictive ability of the linear models which resulted when litters of 2 were excluded. Post-hoc analyses of individual litter sizes indicated that pups from litters of 2 weighed significantly less than those from litters of 3, 4, 5, and 12 and had lower behavioral scores than those from litters of 4, 5, and 6. The remainder of the results are therefore presented with litters of 2 excluded from the analyses.

**TABLE 2. Regression Analyses of Pup Development on Litter Size on Day 32 Post-conception in B6D2F<sub>2</sub> Mice.**<sup>a,b,c</sup>

Developmental Measure (Y)	Regression Model	R <sup>2</sup>	F(model)	df	P
Body weight (g)	$Y = 11.06 - 0.441X$	0.91	185.98	1,18	<0.0001
Brain weight (g)	$Y = 0.45 - 0.00403X$	0.39	11.32	1,18	<0.0035
Thickness of EGL ( $\mu\text{m}$ )	$Y = 13.0 - 0.0288X$	0.003	0.06	1,18	>0.81
Behavioral score	$Y = 0.85 - 0.00705X$	0.35	9.64	1,18	<0.007

<sup>a</sup> The pup data were analyzed by collapsing across sex and using the litter mean score as the unit of analysis.

<sup>b</sup>  $X$  = litter size;  $Y$  = developmental measure.

<sup>c</sup>  $n = 20$  litters. Litter size > 2.

The results of regression analyses on litter size are presented in Table 2. There was a significant negative linear relationship between litter size and (i) body weight, (ii) brain weight, and (iii) behavioral development, but no significant relationship between litter size and EGL. Incorporation of a quadratic component did not contribute significantly to the model. As determined by the correlation coefficients ( $R^2$ ), litter size accounted for 91% of the variance in body weight, but this fell to 39 and 35% for brain weight and behavioral development, respectively.

When the behavioral score was regressed separately on each of the morphological measures, again there was no significant quadratic contribution to any of the models, thereby providing no support for threshold effects.

These relationships (Table 3) were strong for both body and brain weight, with  $R^2$  values of 0.50 and 0.49 respectively. Although the relationship with the thickness of the EGL was less clear ( $R^2 = 0.17$ ,  $0.07 > p > 0.06$ ), it was negative as predicted. As can be seen from the correlation matrix in Table 4, brain weight also correlated negatively with the thickness of the EGL, but despite a positive correlation between body and brain weight, there was no relationship between body weight and the thickness of the EGL.

**TABLE 3. Regression Analyses of Pup Behavioral Score on Morphological Growth on Day 32 Post-conception in B6D2F<sub>2</sub> Mice.<sup>a,b,c</sup>**

Morphological Measure (X)	Regression Model	R <sup>2</sup>	F(model)	df	P
Body weight (g)	$Y = 0.65 + 0.0183X$	0.50	18.14	1,18	<0.0005
Brain weight (g)	$Y = 0.26 + 1.2828X$	0.49	16.98	1,18	<0.0006
Thickness of EGL (μm)	$Y = 0.92 - 0.00977X$	0.17	3.77	1,18	0.07 > p > 0.06

<sup>a</sup> The pup data were analyzed by collapsing across sex and using the litter mean score as the unit of analysis.

<sup>b</sup> X = morphological measure; Y = behavioral score.

<sup>c</sup> n = 20 litters. Litter size > 2.

**TABLE 4. Correlation Matrix of Developmental Measures on B6D2F<sub>2</sub> Mice Pups on Day 32 Post-conception.<sup>a,b</sup>**

	Behavioral Score	Body Weight	Brain Weight	EGL	Litter Size
Behavioral score					
Body weight	0.71 <sup>c</sup> 0.0005 <sup>d</sup>				
Brain weight	0.70 0.0006	0.75 0.0002			
EGL	-.42 0.07	-0.12 0.61	-.45 0.04		
Litter size	-.59 0.006	-.95 0.0001	-.62 .004	-0.06 0.81	

<sup>a</sup> Data were analyzed by collapsing across sex and using the litter mean score as the unit of analysis.

<sup>b</sup> n = 20 litters. Litter size > 2.

<sup>c</sup> Pearson correlation coefficient.

<sup>d</sup> p values.

Table 5 presents the developmental data from the standardized litters at Days 31, 32, and 33. An analysis of variance on these data indicated a significant effect of age on both behavioral score ( $F(2,3) = 20.46$ ,  $p < 0.02$ ) and thickness of the EGL ( $F(2,3) = 16.58$ ,  $p < 0.03$ ). Thirty-one-day-old animals scored significantly lower than both 32- and 33-day-old animals on the behavioral score and their EGL was thicker than that of 33-day-old animals; both these effects were in the direction expected for age. There were no significant effects of age on body or brain weight. This was not unexpected given the narrow age range combined with the fact that, when the scale was developed initially, these latter two measures showed larger variability and less predictability across age than did the EGL and behavioral development.

**TABLE 5. Development of B6D2F<sub>2</sub> Mice as a Function of Age Post-conception.<sup>a,b,c</sup>**

Age (days)	Body Weight (g)	Brain Weight (g)	EGL Thickness (μm)	Behavioral Score
31	7.4 ± 0.1	0.416 ± 0.017	17.8 ± 1.3	0.62 ± 0.04
32	7.1 ± 0.1	0.411 ± 0.003	14.5 ± 0.5	0.79 ± 0.02
33	7.6 ± 0.3	0.433 ± 0.011	11.0 ± 0.5	0.84 ± 0.003

<sup>a</sup> Data are presented as means ± SEM.

<sup>b</sup> Data were analyzed by collapsing across sex and using the litter mean score as the unit of analysis.

<sup>c</sup> n = 2 litters per age.

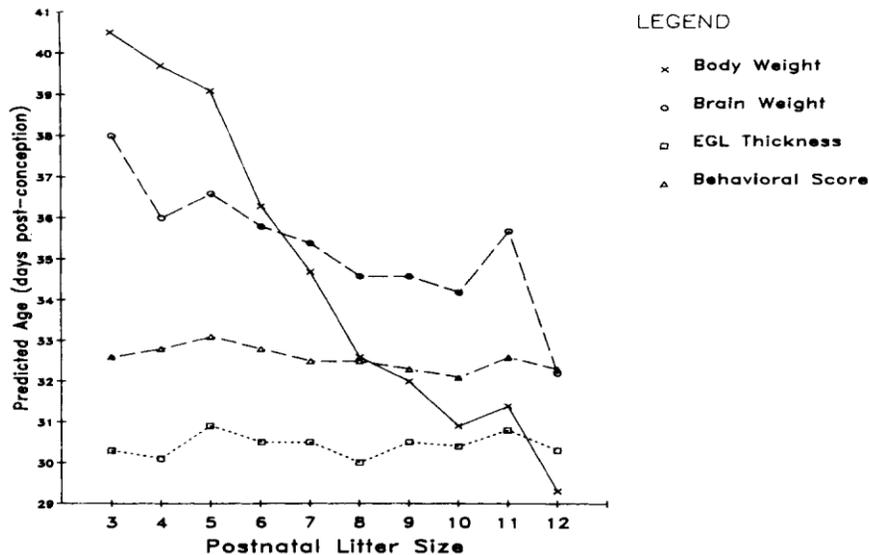


Fig. 1. Relationship between litter size and development of B6D2F<sub>2</sub> pups on Day 32 post-conception. Predicted ages were obtained by substituting litter mean values for each variable into previously derived regression equations (described in the text)

The relative magnitudes of the effects of litter size on the four measures are evident from plots of predicted developmental ages shown in Figure 1. Retardation of growth by increasing litter size was much greater for the entire body than the brain, whereas effects on EGL thickness and behavioral score were quite small. An index of growth retardation was derived for each measure by using the regression equations in Table 1 to determine expected values for litters of 3 and 12, and then inserting these values in the quadratic equations from the time scale to determine expected developmental age. For example, the expected body weight of a litter of size 3 is 9.74 g, which is the expected weight of the standard B6D2F<sub>2</sub> mice at 40.85 days from conception, and for litter size of 12 the expected weight of 5.77 g corresponds to standard B6D2F<sub>2</sub> mice at 29.31 days. Thus, litters differing by 9 pups differ in body weight by an amount equivalent to 11.54 days of standard growth, which is about 1.28 days retardation of growth for every additional pup in the litter. For brain weight, litters of 3 and 12 differ by 36.3 mg, which is a difference of 3.93 days in standard growth, or 0.44 days retardation per additional pup. Consequently, retardation of brain growth (0.44 day/pup) is much less than whole body growth (1.28 day/pup) at 32 days post-conception, which demonstrates brain sparing. The difference in behavioral score for litters of 3 and 12 was 0.063 units, equivalent to a difference of 0.60 days in age, or 0.07 day per pup, which shows that the degree of retardation of reflex ontogeny by larger litters was much less than for brain growth. For EGL thickness, there was scarcely any effect of litter size (0.01 day/pup).

## Discussion

These results indicate that manipulation of preweaning litter size in B6D2F<sub>2</sub> mouse pups was effective in producing a continuous range of variation in overall growth, as measured by body weight. The relationship between litter size and body weight was best described by a linear regression model and was negative, in that an increase in litter size correlated with a decrease in body weight. There was a similar, although less powerful, effect on brain weight, but the growth of the cerebellar EGL appeared to be unaffected by differences in litter size. The effect on brain weight is supported by a recent study of a litter size effect on adult brain weight in BALB/c mice (Wahlsten & Bulman-Fleming, 1987). When behavioral development was regressed on each of these morphological measures the relationship was again linear and positive in the case of body and brain weight and negative for the EGL. This relationship between body and brain growth and behavioral function supports a continuity of effects on growth and function, rather than a threshold model.

It is interesting to note that although neither litter size nor body weight was correlated with the thickness of the EGL, both brain weight and behavioral development were negatively correlated with this measure. Given that the measures on the standard litters showed that both the EGL and behavioral score changed predictably with age, thereby supporting the sensitivity of these measures, these results imply that some aspects of brain growth may be relatively independent of nutritional effects on overall body growth. Although previous work has shown

that undernutrition does affect the growth of the EGL and appears to retard the disappearance of these cells (Altman & McCrady, 1970; Barnes & Altman, 1973a,b; Lewis, Balazs, Patel, & Johnson, this may depend on the severity of the treatment (Barnes & Altman, 1973a). A study which did show effects of large litter rearing on various other aspects of cerebellar growth (Neville & Chase, 1971) did not find alterations in the migration of granule cells from the external to internal granular layer, which may be related to the degree of undernutrition produced by the manipulation of litter size. There is a concern with the present data in that the thickness of the EGL in the present study was slightly larger than the values that reported previously and therefore resulted in predicted ages lower than the actual age. As the histological procedures were similar between the two studies, this may be an indication of sampling error. Comparison of equivalent litter sizes at the same age between the studies support this by also showing slight increases in both body and brain weight in the present study.

Despite the linear relationship between growth decrement and behavioral function, when these measures were converted to a common scale it was readily apparent that there was a large difference in the relative magnitude of the morphological compared with the behavioral effects. For example, the predicted ages showed that an increase of one pup in a litter decreased body weight by an equivalent of 1.28 days, whereas the similar figure for behavioral development was only 0.07 day. It should be noted that the ages predicted from the behavioral scores were very close to the chronological age. This accuracy is particularly impressive in view of the fact that not only was evaluation of individual pups done blindly, but additional measures were taken to ensure that not all animals from the same litter were tested sequentially in order to minimize any bias due to expectations derived from prior testing of that litter. In addition, the fact that behavioral scores increased predictably with age, without any significant differences in body weight over the narrow age range, speaks against the possibility that the scoring procedure was being influenced significantly by the size of the animal. These results therefore support the interpretation of considerable sparing of behavioral function relative to effects on overall growth.

There are some caveats with respect to this interpretation. One is that the range of litter sizes used here did not encompass extreme undernutrition and that there may indeed be a point beyond which the sparing of behavior is no longer possible. Certainly the effect on behavioral development of rearing in litters of 12 compared with litters of 8 (0.2 days) was small relative to previous work which showed an effect of prenatal protein malnutrition of 1.4 days (Wainwright & Russell, 1983). However, in support of the present data, there were large effects on overall growth, and recent work in our laboratory (Wainwright & Francey, 1987) has shown permanent deficits in adult body weight in this population of mice consequent on rearing in litters of 12, suggesting that they were indeed undernourished. It is also important to recognize that there may be behavioral measures other than those used here which are more sensitive to nutritional effects, and this should be addressed in future research. Another concern is that manipulation of litter size affects not only pup nutrition but also the nature of the interaction between dam and offspring (Plaut, 1970; Priestnall, 1972; Crnic, 1980), and presumably between offspring themselves. It is interesting to note that, although pups reared in litters of 3 were the heaviest in the study, those reared in litters of 5 had the most advanced behavioral score. One might speculate that differences in social interactions are contributing to such findings.

In summary the results of this study show that, within the range of nutrient availability resulting from the manipulation of litter size, effects on the behavioral development of preweaning mouse pups was linearly related to effects on body and brain growth, with no evidence of a threshold effect. Nevertheless, the magnitude of such effects on behavior were considerably smaller than those on the morphological measures. These findings demonstrate that although behavioral development is sensitive to variability over the complete nutritional range, there nevertheless appears to be considerable sparing of behavioral function when compared with effects on overall growth.

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