Effects of Undernutrition at Different Ages Early in Life and Later Environmental Complexity on Parameters of the Cerebrum and Hippocampus in Rats[1]

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ABSTRACT Nutritional deprivation at different stages of development in rats was shown to cause reductions in some parameters of the cerebrum that could not be reversed by an extended period of adequate feeding. The deficits varied in magnitude, depending on whether undernutrition occurred during the suckling period alone or was combined with additional deprivation either before birth or after weaning. Whereas the weight, length, and width of the cerebrum were affected significantly by undernutrition at every age, effects on the thickness of the cortex and hippocampus were associated only with the combined pre- and postnatal deprivation. The deficits in weight, length, and cortical thickness could be modified later by housing in enriched and impoverished environments for 30 days. The previously undernourished rats responded similarly to environmental complexity regardless of the age at which they had been deprived, and their responses did not differ significantly from those of well-fed controls. The degree to which enrichment can be said to have reduced deficits arising from undernutrition depends on the relative size of the nutritional and environmental effects on the particular parameters in question and on the choice of a "normal" baseline against which to assess recovery. J. Nutr. *112:* 1362–1368, 1982.

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A number of investigations have shown that reductions in the behavioral or intellectual competence of malnourished infants may be due in part to the inadequate amounts of social and psychological stimulation they receive (1, 2). There is evidence that the recovery of function can be enhanced by improving the environment in those respects (3–6). These, and similar findings with laboratory animals (7–9), have led us to question whether environmental factors might also contribute to the effects of malnutrition in early life on the brain. Our earliest experiment on the subject measured the effects of environmental complexity on the brain weight of rats that had been undernourished during the suckling period. We found that the lasting deficit associated with the undernutrition could indeed be modified by a 30-day period of enriched and impoverished experience subsequently (10).

These findings are confirmed and extended in the present experiments. In the first, undernutrition was again imposed during the suckling period, while in the second and third, further deprivation took place either before birth or after weaning. In each case, there was an extended period of nutritional rehabilitation before the rats were exposed to differential environments. Imme-

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diately afterwards measurements were made of the weight and size of the cerebrum and of the thickness of the cerebral cortex and hippocampus. It was considered likely that some of these parameters would reflect an interaction between nutrition and environment on the brain because their susceptibility to one or the other factor had been established in previous research (11–14).

Although the chief objective of the investigation was to gain information on the combined effects of undernutrition and environmental complexity on the brain, it was also possible to compare their separate effects and to show that morphological features of the brain may differ in susceptibility to those conditions. Since the experiments were conducted independently, and involved inevitable differences in methodology, it was not possible to make formal comparisons between the effects of undernutrition at different ages.

METHODS

Nutrition

Experiment 1. Lactational undernutrition. At parturition, litters of black and white hooded Lister rats, derived from the departmental colony, were culled to 8 young with a maximum of 6 males. From the day of birth to 21 days of age, the offspring were undernourished by restricting the food intake of their mothers, which had previously been fed normally, to about half the amount of stock diet[3] eaten ad libitum by well-fed controls. This feeding schedule markedly reduces lactation, thereby limiting the supply of milk to the young (15) during a time when the brain is developing rapidly and is highly vulnerable to undernutrition (16). Food was made freely available to both mother and offspring on day 21; however, to ensure the survival of the stunted offspring, weaning did not take place until a week later. At that time, the male offspring were rehoused in groups of 3–5 in plastic cages (25 × 40 × 20 cm), which contained wooden shavings. They continued to receive unrestricted amounts of food and water until killed at about 6 months of age.

Experiment 2. Gestational and lactational undernutrition. From the day of conception until parturition, pregnant rats of the same strain as above were given daily rations of stock diet consisting of about half the amount eaten by controls. At birth the litters were culled as previously described. The food intake of underfed mothers continued to be restricted throughout the lactation period. When the young were 25 days old, food was supplied ad libitum. They were weaned on day 30, when male offspring were placed in groups of 2–3 in standard laboratory cages. They continued to have free access to food and water for the remainder of the experiment.

Experiment 3. Lactational and postweaning undernutrition. Undernutrition was imposed continuously from the day of birth to 42 days of age by restricting the food intake of the mothers during the suckling period as above and that of the young after weaning. The length of deprivation in this experiment was similar to that in experiment 2 in which gestational and lactational undernutrition were combined. Every litter, including the controls, contained 8 rats with a maximum of 6 males. At weaning on day 25, the males from the same litter were housed in groups of 2–4 in standard cages. From 43 days until the end of the experiment the rats were fed and given water ad libitum.

Environment

At approximately 5 months of age in every experiment littermate pairs of male rats, which had been previously earmarked for identification, were selected from available litters on the basis of similar body weights. They were then assigned randomly to either enriched or impoverished environmental conditions for 30 days (14). The enriched rats were housed socially in groups of 12–16, depending on the experiment, in wire cages (45 × 65 × 36 cm), which contained about 10 objects from a collection of miscellaneous items, such as pieces of wood, metal cage tops, brushes and bottles. (In experiment 1 the enriched cages were double-sized, but contained the same density of objects.) Some of these were suspended from the ceiling of

[3] Porton mouse diet, RHM Labsure Ltd., Poole, Dorset, England; percentage by weight: carbohydrate, 53.5; protein, 19.8; fiber, 5.4; oil, 2.8; moisture, amino acids, vitamins and minerals, 18.5.

the cage with wire supports to allow climbing and vertical exploration. Food and water were available at several locations in each cage. Throughout the period of enrichment, normally nourished and previously undernourished rats were kept in different cages. Every day, near the end of the white phase of the 12-hour-red/12-hour-white light cycle, the groups were placed into a cage that contained an arrangement of objects they had not previously experienced. In all cases impoverished rats were housed singly in standard plastic cages in the same room as their enriched littermates. They too ate and drank ad libitum. Room temperature was maintained at approximately 23°.

Brain measurements

After differential experience, the rats were killed by chloroform anesthesia in random order. The experimenter was not aware of their identity. Brains were removed, then with transverse cuts at the level of the anterior and posterior poles, the olfactory bulbs, cerebella and medulla were detached. The remaining brain, which will be referred to subsequently as cerebrum, was weighed immediately and fixed in 10% buffered formalin. After fixation, the samples were rinsed in phosphate buffer.

To obtain length and width measurements each brain was placed ventral surface down on a Zeiss microprojector (Carl Zeiss, Jena, E. Germany), and its magnified (×13) image was traced. The length was measured along the midline between two lines tangent to the anterior and posterior poles of the cerebral hemispheres. Width was measured perpendicular to the length component at the point of maximal extent. Measurements of the thickness of the medial occipital cortex and the underlying hippocampus were made from drawings of the projected images (×26.5) of coronal sections, which were matched according to the presence of the habenular nucleus and posterior commissure and stained with cresyl violet. See Katz and Davies (17) for a diagram of this section and detailed histological procedures. Cortical thickness represents the mean distance between the dorsal surface of the corpus callosum and layer 2 of the cortex (or the pial surface in experiment 1). Measurements were made in both hemispheres perpendicular to the tangent of the pial surface at 5 equidistant points lateral to the elevation of the corpus callosum in the cortex and at 2 points at the medial and lateral edge of the stratum granularis in the hippocampus.

Statistical analyses

Raw data were analyzed individually for each experiment according to a three-factor analysis of variance, nested design (18). The factor *litter* is nested under *nutrition* because previously undernourished and control rats were necessarily drawn from different litters. Both, however, are crossed with *environment* since offspring from every litter and both nutritional backgrounds were either environmentally enriched or impoverished. The error term for *nutrition* is *litter w. nutrition*, whereas that for *environment* and the interaction between *environment* and *nutrition* is *(litter w. nutrition)* × *environment*.

RESULTS

Experiment 1. Lactational undernutrition

On day 21 when the period of restricted maternal food intake ended, undernourished offspring weighed on average 15.9 g in contrast to those from well-fed litters, which weighed 39.9 g (61% deficit; $t(1/23 \text{ litters}) = 27.9$, $P < 0.001$). After about 6 months of unrestricted feeding, the previously undernourished males still showed a deficit in body weight of 17.3%. There were also significant reductions of about 2–6% in the weight, length and width of the cerebrum as a result of the lactational undernutrition (table 1), but no long-term deficits in the thickness of the cortex (see also ref. 19) or the hippocampus were observed.

Subsequent housing in differential environments for 30 days resulted in the impoverished rats weighing about 7% more than their enriched littermates ($P < 0.0001$). The interaction term showed a tendency for the environmental effect on body weight to be greater among previously undernourished rats than controls ($P < 0.1$). The effects of differential housing on the weight, length and width of the cerebrum were significant, but they were somewhat smaller than those

TABLE 1

Percentage effects of nutritional and environmental treatments

Experiment[1]	Period of undernutrition[2]	Nutrition		Environment			Interaction
		E + I[3]	P[4]	C[5]	PU[5]	P[4]	P[4]
Final body weight							
1	L	−17.3	<0.0001	−4.2	−10.0	<0.0001	<0.1
2	GL	−29.6	<0.0001	−10.1	−15.0	<0.0001	ns
3	LW	−12.2	<0.0001	−1.8	−6.2	ns	ns
Cerebral weight							
1	L	−5.8	<0.0001	3.7	4.1	<0.001	ns
2	GL	−14.4	<0.0001	2.9	1.8	<0.01	ns
3	LW	−4.1	<0.01	4.3	1.2	<0.025	ns
Length							
1	L	−2.1	<0.01	2.7	1.1	<0.005	ns
2	GL	−5.0	<0.0001	0.8	1.6	<0.025	ns
3	LW	−1.7	<0.01	1.4	1.3	<0.005	ns
Width							
1	L	−3.2	<0.001	1.9	0.5	<0.02	ns
2	GL	−5.0	<0.0001	1.8	−0.2	ns	<0.1
3	LW	−3.0	<0.005	0.1	1.5	ns	ns
Cortical thickness							
1	L	−0.5	ns	5.2	4.9	<0.0001	ns
2	GL	−5.9	<0.0005	6.4	8.6	<0.0001	ns
3	LW	−3.4	<0.1	5.6	3.0	<0.0001	ns
Hippocampal thickness							
1	L	−0.7	ns	1.6	1.2	<0.1	ns
2	GL	−3.4	<0.005	−0.2	−0.5	ns	ns
3	LW	−2.0	<0.1	0.8	0.4	ns	ns

[1] Experiment 1, n = 12 pairs; experiment 2, n = 15 pairs; experiment 3, n = 12 pairs. [2] G, gestation; L, lactation; W, postweaning. [3] E, enriched; I, impoverished; % environmental effect = (E − I)/I × 100. [4] Probability values refer to the main effects of nutritional and environmental treatments and their interaction as given by analysis of variance, nested design. Values up to $P < 0.1$ are given in order to indicate the extent to which differences were statistically significant; ns = not significantly different. [5] C, control; PU, previously undernourished; % nutritional deficit = (PU − C)/C × 100.

produced by the undernutrition. The opposite was true for cortical thickness, however, which was altered more by environment than nutrition. The environmental treatment did not significantly affect the thickness of the hippocampus. No differences could be detected in the responses of the previously undernourished and well-fed rats to differential environments; that is, there was no significant interaction effect between *nutrition* and *environment* for any brain parameter.

Experiment 2. Gestational and lactational undernutrition

Maternal underfeeding during gestation reduced fetal growth substantially. At birth, the average body weight of the male offspring in the undernourished litters was 4.5 g relative to 5.8 g for the controls (22%, t (1/20 litters) = 9.3, $P < 0.001$). By the end of the continued underfeeding during the suckling period, they weighed, respectively, 17.6 g and 56.3 g, which represents a 69% deficit (t (1/20 litters) = 35.8, $P < 0.001$). The deficit in body weight remained about 30% after nearly 5 months of unrestricted feeding.

There were marked reductions in parameters of the brain as a result of the pre- and postnatal undernutrition. The deficit in cerebral weight at 6 months of age was approximately 14% and that of length and width were 5% each. Cortical and hippocampal thickness, which were not reduced significantly in the previous experiment involv-

ing lactational deprivation alone, showed lasting deficits of 5.9% and 3.4%, respectively, for combined gestational and lactational undernutrition (table 1).

In relation to the other experiments, there was a larger deficit in the body weight of enriched relative to impoverished rats. The difference could be related to the extra number of rats that were present in the enriched conditions in this experiment (several more per cage than in experiments 1 and 3). Competition for food may have been increased, as aggressive activity was noted particularly among the previously undernourished rats (20). Despite their decreased body weight, environmentally enriched rats still showed increases relative to impoverished littermates in most parameters measured in the brain. Except for cortical thickness, these increases were smaller than the deficits associated with undernutrition. Differential experience did not significantly affect either the width of the cerebrum or thickness of the hippocampus. The size of the environmental effects on previously undernourished and control rats (i.e., the interaction effects) was not significantly different for any parameter, although the width of the cerebrum showed a tendency to be affected less by environment in undernourished as opposed to well-fed rats.

Experiment 3. Lactational and postweaning undernutrition

At the end of the period of undernutrition at 42 days of age, the average body weight of offspring from undernourished litters was 41.7 g relative to 145.7 g for the normally nourished controls (70% deficit; t (1/27 litters) = 60.5, $P < 0.001$). The deficit had fallen to about 12% by the time the rats were killed at about 6 months of age (table 1). Significant deficits were found in the weight, length, and width of the cerebrum as a result of the extended period of postnatal deprivation. Deficits in the thickness of the cortex and hippocampus were marginally significant.

Even though the duration of undernutrition and its effect on body weight was approximately the same as in experiment 2 on gestational and lactational undernutrition, and the length of nutritional rehabilitation shorter, the effects of nutritional deprivation on the brain were less pronounced. This could be due in part to an enhancement in the severity of the deprivation during lactation in experiment 2 because the mothers had been previously deprived during gestation (21, 22).

The difference in the body weight of enriched and impoverished littermates was not statistically significant. Significant effects of environment were observed on the weight and length of the cerebrum and on the thickness of the cortex, but not on width or hippocampal thickness. The effects of differential housing on previously undernourished and control rats were not distinguishable according to the interaction terms for the brain parameters.

DISCUSSION

Undernutrition during the lactation period (experiment 1) or with further deprivation imposed during gestation (experiment 2) or after weaning (experiment 3) caused lasting deficits in some, but not all, of the parameters of the cerebrum that were measured. In all three experiments reductions were found in the weight and size of the cerebrum (see also refs. 12, 23), but significant deficits in the thickness of the cortex and hippocampus were only associated with combined gestational and lactational undernutrition. The pre- and postnatal undernutrition also apparently produced larger deficits than did exclusively postnatal deprivation. This is probably not a result of the longer duration of deprivation because undernutrition of similar length during lactation and after weaning did not have equivalent effects. Since lactational undernutrition was common to both, the experiments suggest a relatively greater effect of nutritional deprivation before birth in rats than after weaning. Continued deprivation after weaning, however, did seem to add to the effects that were produced by lactational deprivation alone.

It is important to realize that definitive conclusions about the relationship between the timing of undernutrition and its effects on the brain cannot be made from these experiments. There is no way in this or any study to be sure that the methods for undernourishing at different stages of development

produced deprivation of comparable severity, even though criteria for the amount of maternal food restriction or proportional body growth restriction in the young may be established. Furthermore, since the present experiments were conducted at different times, unknown (and some known) procedural differences are likely to have existed, so that statistical tests that could formally compare their results were inappropriate.

Like undernutrition, differential housing in enriched and impoverished environments for 30 days did not affect the brain uniformly. The largest effects of environmental complexity were on the thickness of the cortex (24), while small but still significant changes were also found in the weight and length of the cerebrum. In agreement with most other reports in the literature (25), significant effects of differential housing on the width of the cerebrum (except in experiment 1) and the thickness of the hippocampus were not detected.

The effects of environmental complexity on previously undernourished and well-fed rats could not be distinguished, regardless of when early in life the nutritional deprivation had been imposed. That is, according to the measurements we have undertaken so far, the brain's response to differential environments was not altered significantly by the lasting effects of early undernutrition. Such an alteration might have been expected for at least two reasons. First, it is possible that specific anatomical or physiological substrates in the brain on which normal environmental effects depend would be disrupted by nutritional deprivation during vulnerable periods of development. Second, there is evidence that unless motivated by food reward, malnourished animals may be deficient in attending to or learning about their surrounding environment (26–28). Because our preliminary measurements are fairly coarse and because the environmental effects in any case are small and variable, we believe that it would be premature to dismiss the possibility that undernutrition might alter cerebral responses to environmental complexity. In this (cerebral width in experiment 2) and other experiments (10) we have occasionally found statistical interaction effects that approached significance and would have indicated a diminution in the response of previously undernourished groups to differential environments.

Some morphological parameters of the brain were affected both by undernutrition early in life and by environmental complexity later. Others, however, were affected significantly by one but not by the other condition. Obviously the possibility of modifying nutritionally induced deficits by environmental means is limited to that category of parameters that comprises the overlap between the effects of the two conditions. With little or no effect of differential environments on the width of the cerebrum and the thickness of the hippocampus, deficits in those parameters from early undernutrition remained more or less fixed. In contrast, deficits in the weight and length of the cerebrum and in cortical thickness were modified significantly by manipulating environmental complexity. In some cases the effects of the differential environments matched or even exceeded the magnitude of the lasting effects of undernutrition.

Although it is generally arbitrary to define a "normal" baseline when considering environmental complexity, there are in malnutrition circumstances which are likely to involve real or functional reductions in the amount of psychological stimulation received (1, 28). Therefore, it is not unreasonable in this context to consider enrichment as a special treatment for malnutrition (3–6) and to use impoverished conditions as the point of comparison. If so, enrichment can be seen to have improved values of some cerebral parameters that had been reduced by undernutrition early in life, but only if the surrounding environment was less than optimal. In some cases, enriched undernourished rats had values that were significantly greater than those of impoverished littermates and that approached levels of impoverished, well-fed controls. (It should be stressed, however, that the trade-off between nutritional and environmental factors was observed on gross dimensions of the brain and may not represent the effects on specific elements). Yet, in every case they failed to reach levels that were attained by well-fed rats that also had been exposed to an enriched environment. From this viewpoint, enrichment may have

been a benefit, but it did not act as a remedy for early undernutrition since residual deficits were still visible. Finally, it should be said that it is not yet known whether the environmentally mediated changes are linked to reported functional improvements in undernourished animals (7–9) or how long they would persist if the rats were once again returned to standard housing conditions.

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