LASTING EFFECTS ON MOUSE BRAIN GROWTH OF 24 HR POSTPARTUM DEPRIVATION

By: Douglas Wahlsten, Kathryn Blom, Roswitha Stefanescu, Kent Conover, and Heather Cake

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Abstract:
When inbred BALB/c mice were separated from their mother for 24 or 36 hr beginning shortly after birth, growth of the body, whole brain and corpus callosum was almost completely stopped. After being returned to their mother, mice deprived for 24 hr gained weight more slowly than non-deprived littermates over the next 6 days but later showed moderate catch-up growth after weaning at 4 weeks of age. After 55 days of recovery, mice deprived for only 24 hr showed significant reductions in brain weight and size of forebrain commissures compared to littermate controls. Approximately twice as many deprived mice had a corpus callosum that was abnormally small compared to controls. These results demonstrate that a rather brief but severe period of separation from the mother can have lasting effects on brain growth.
Key words: Corpus callosum, Anterior commissure, Brain weight, Inbred strain, Nutrition

Article:
Virtually all animal models of human undernutrition[6] have used several consecutive days of inadequate nutrition produced by low protein diets,[11] low amount of a normal diet,[3] large litter size,' or separation from the mother for several hours each day.[10] One could easily gain the impression from this research that only a period of several days deprivation will have lasting effects on brain growth and that recovery from a short but severe period of starvation will be complete. However, effects of very short-term starvation have received little attention from researchers interested in animal models, and it is not at all clear whether there is a lower threshold for producing long-term deficits in brain growth by early undernutrition.[19]

This problem came to our attention while observing the maternal behavior of inbred mice. It is known that care of the young is generally poor in inbred compared to hybrid mice.[18] We noticed that inbred mothers often neglect their young for several hours or even a day after parturition and that full lactation often does not commence until the day after birth. Consequently, experiments were done to assess the impact of this kind of brief starvation on brain growth and recovery from starvation.

The strain chosen for these experiments, BALB/c, is known to have hereditary absence or deficiency of the corpus callosum.[14] The defect is known to be multifactorial rather than the result of a single gene,[15] and its severity and frequency can vary according to the environment.[14,16] Thus the experiments also examined whether postpartum starvation, which occurs at the time of most rapid corpus callosum growth,[17] would increase the frequency or severity of overt defects in adult mice.

First a study was done to verify that starvation after birth does indeed arrest brain growth and then two studies were done to assess recovery after starvation of either an entire litter or only one or two pups in a litter. Preliminary tests compared newborn pups separated and maintained at either room temperature or 37°C. Although the latter procedure prevented hypothermia, it led to serious dehydration and frequent mortality after 24 hr. For this reason, and because neglect of pups by the mother does in fact produce hypothermia, all experimental animals were isolated at room temperature in all studies.

EXPERIMENTAL PROCEDURES

Animals

Mice of the inbred BALB/cCF strain were maintained under standard conditions[14,15] and given free access to Master MLM Rodent Chow. All matings were between brother and sister. The day of birth was regarded as day 0P.

Immediate effects of separation

Pregnant mice were observed at least twice daily for births and litters of at least six pups were chosen for study after the mother had cleaned them and gathered all into the nest. Each pup was weighed to the nearest mg and then marked with a blue felt pen. Weights of all mice in the litter were examined and a 'triad' of animals closely matched with respect to birth weight, presence of milk in the stomach, and, where possible, sex was chosen for study. In litters of 6-8 pups only one triad was used, whereas two triads were chosen from litters of 9 or 10 pups. Within each triad, one pup was perfused with 10% formalin immediately (0 hr) using methods described previously,[17] one was isolated in a dish containing clean tissue paper at room temperature (22°C) for either 24 or 36 hr and then perfused, and the third one remained with its mother and other siblings for either 24 or 36 hr and was then perfused at the same time as its deprived littermate. There were 9 triads in the 24 hr condition and 10 in the 36 hr condition. Brains were weighed and encased in gelatin, and then sagittal sections were cut, stained and measured as described previously.[17]

Recovery after deprivation of whole litter

Only litters of 3 or more pups were used in this phase. In certain litters all pups were marked, weighed and isolated for 24 hr after birth and then returned to their mother until weaning at 4 weeks, whereas mice from closely related control litters were marked and weighed in the same manner but remained with their mothers until weaning at 4 weeks. After weaning, mice were housed with same-sex littermates for 4 more weeks. Recovery was monitored by weighing the mice 24 hr after return to the mother and then weekly until weaning at 4 weeks. At 8 weeks of age each surviving mouse was anesthetized with pentobarbital sodium and perfused intracardially with saline followed by 10% buffered formalin. The brain was subsequently weighed, frozen, cut sagittally at 33 μm and stained with metachromatic thionin or Sudan Black B. Cross-sectional areas of the corpus callosum (CC) and the anterior commissure (AC) were measured at the midsagittal plane.

Recovery after deprivation of matched littermates

All pups were weighed and marked soon after birth as in the whole-litter study, and then within each litter one or two pups near the median body weight for the litter were isolated for 24 hr and then returned to the nest. If the litter had 3-6 pups, only one was deprived, whereas two were deprived in litters of 7 or more. There were 20 litters in the study at the outset but two were excluded because the deprived pup eventually died. Data are reported for 24 deprived and 24 non-deprived littermates matched for sex and birth weight. These mice were reared and tested the same way as those in the whole-litter study.

RESULTS

Immediate effects of separation

Figure 1 presents mean values for mice in the triads, including non-deprived mice at three ages as well as for mice deprived for 24 and 36 hr. Because there were no significant differences (all $P>0.40$) between 0 hr control mice in the 24 hr and 36 hr triads, their data were combined to yield a single mean value at 0 hr in Fig. 1. Obviously growth was rapid in non-deprived animals. The differences between deprived mice and their non-deprived controls which remained with the mother were highly significant for body weight, brain weight and CC area at both 24 and 36 hr (all $P<0.001$ by matched-pairs t-tests). In the 24 hr group the deprived mice lost an average of 36 mg body weight compared to their own 0 hr values, but they increased significantly in brain weight by about 4 mg compared to 0 hr control animals ($t = 3.9$, $P< 0.005$, two-tailed), although this modest brain growth was much less than the 21 mg increase for non-deprived controls over the same period. Brain weights of the 36 hr deprived animals did not differ significantly from 0 hr controls, which indicates that the small 'brain sparing' effect seen in the 24 hr group was of short duration. There were no significant differences between CC areas of 0 hr controls and either 24 or 36 hr deprived mice.

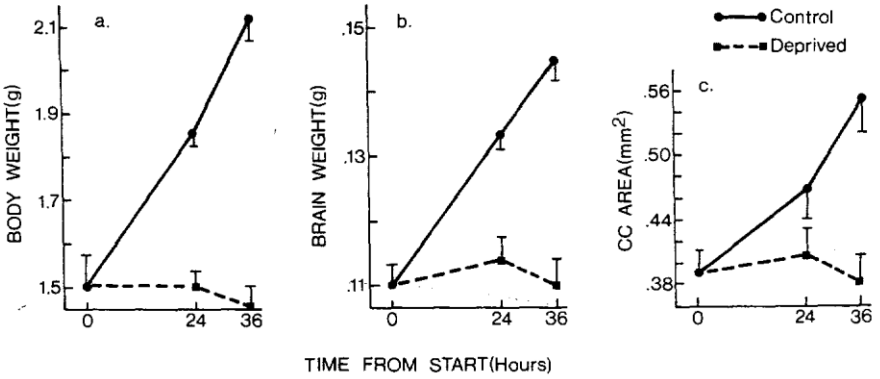

Fig. 1. Mean values for (a) body weight, (b) brain weight and (c) cross-sectional area of the corpus callosum (CC) for mice perfused shortly after birth (0 hr) and their matched littermates which were deprived for 24 or 36 hr or (controls) left with the mother and siblings for the same amount of time and then perfused. Brackets indicate 1 S.E.M.

Weight gain during recovery

The whole-litter study was complicated by high mortality among deprived pups. Of the 85 mice in 11 litters alive at birth, only 46 survived until testing at 8 weeks. Most of them, including two entire litters, perished within a day or two of return to their mothers, because of either cannibalism or total neglect. Among the 8 control litters in the whole-litter study, only 5 of 54 mice died prior to 8 weeks. This differential mortality resulted in smaller litters for previously deprived pups and consequently a somewhat higher rate of growth[2] than if there had been no mortality. Therefore, the data for the whole-litter study were analysed using multiple regression to adjust for effects of litter size and sex differences. The analysis was done on mean body weights of mice of the same sex in each litter rather than individual scores.[1] Separate analyses were done at each age because of large differences in variance. For the whole-litter study means in Table 1 represent the average weight of males and females at a common litter size of 6 pups during the recovery period. Tests of significance are shown for the partial regression coefficient for the deprivation effect. For the within-litter study, mortality was low and analysis was done with simple matched-pairs t-tests.

Table 1. Mean body weights (g) of control and deprived mice

	Whole-litter study			Within-litter study		
	Control	Deprived	*t*	Control	Deprived	*t*
Litters	8	9		18	18	
Mice	48	46		24	24	
Age:						
0	1.40	1.39	0.24	1.43	1.43	0.22
1	1.62	1.36	4.12*	1.71	1.40	12.63*
2	1.96	1.39	7.33*	2.05	1.60	15.19*
7	4.68	3.63	7.44*	4.78	4.02	11.89*
14	7.78	6.93	2.97*	7.53	6.88	9.95*
21	8.91	8.01	2.56*	8.84	7.89	11.66*
28	11.92	10.52	2.60*	12.71	11.42	6.79*
56	19.88	19.20	1.19	20.98	20.11	3.76*

*$P<0.05$, one-tailed.

In the whole-litter study, weight gain was less in deprived than control litters during the 24 hr after return to the mother (t = 9.39, $P<0.001$) and from day 2 to day 7 (t = 5.52, $P<0.001$). No group differences in weight gain were evident from day 7 to weaning at day 28, but in the 4 weeks after weaning the previously deprived mice gained weight slightly more rapidly than controls (t = —2.38, P= 0.01). In the within-litter study a similar pattern occurred. Weight gain of controls Was significantly greater than matched deprived pups from day 1 to 2 (t = 6.88, $P<0.001$) and day 2 to 7 (t = 5.67, $P<0.001$) but not day 7 to 14 ($P> 0.2$). Controls also grew faster from day 14 to 21 and day 21 to 28 (both $P< 0.05$), but deprived pups grew faster than controls in the 4 weeks after weaning (t = —2.35, $P< 0.05$). Comparing the deprived mice in the two studies, those in the within-litter condition gained weight faster during the 24 hr after return to the mother than did those in the whole-litter study (t = 5.44, $P<0.001$) but thereafter the differences in weight gain were relatively small.

It is noteworthy that weight differences at 8 weeks between control and deprived mice (Table 1) were similar in the whole-litter study (0.68 g) and within-litter study (0.87 g) but that only the latter was statistically significant.

Table 2. Mean values at 8 weeks after birth for two groups of mice

Measure	Control	Deprived	Difference	*t*
Whole-litter study				
Sample size	47	46		
Brain weight (mg)	486.6	475.5	11.1	1.97*
AC area (mm^2)	0.133	0.130	0.003	1.26
CC area (mm^2)	0.951	0.907	0.044	0.75
No. of abnormal CC	6	11		
Within-litter study				
Sample size	24	24		
Brain weight (mg)	501.5	485.8	15.7	4.41‡
AC area (mm^2)	0.132	0.127	0.005	2.72*
CC area (mm^2)	0.994	0.834	0.160	3.64†
No. of abnormal CC	2	6		

*$P<0.05$; †$P<0.01$; ‡$P<0.001$.
AC, anterior commissure; CC, corpus callosum.

Brain and fiber tract sizes at 8 weeks

Table 2 presents mean brain weights and cross-sectional areas of fiber tracts for mice at 8 weeks after birth. In the within-litter study the deprived animals had significantly lower values on all measures, using one-tailed *t*-tests. Because small CC area was an infrequent anomaly, use of litter means tended to obscure the presence of an abnormal mouse in a large litter. Consequently, data for the whole-litter study were analysed using individual scores with multiple linear regression to adjust for sex and litter size effects. The effects of deprivation were in the same direction as the within-litter study but reduced in magnitude. Using area less than 0.8 mm^2 as a criterion of abnormally small CC,[15] there were more abnormalities among deprived animals in both studies. Pooling data from the two studies, 24% of deprived mice and 11% of controls showed abnormal CC at 8 weeks, which was a marginally significant difference ($z = 1.81$, $P=0.04$, one-tailed).

DISCUSSION

When littermate controls are compared to mice deprived of contact with their mother for 24 hr starting on the day of birth, lasting effects of deprivation on body, brain and corpus callosum size are evident 8 weeks later. The effect on body size is not large, especially compared with those of a low protein diet during the lactation period which reduces mouse body weight anywhere from 6.5 g or 14% at 80 days of age to 24% at 500 days. [13] The 15 mg reduction in brain weight is more substantial but is still less than the deficit produced by daily separation from the mother,[8] large litter size [12] or low protein diet[4,13] during lactation, which reduce adult brain weight by 20-40 mg. The magnitude of the effect on brain weight is similar to that of postnatal environmental enrichment in mice.[7] In BALB/c mice the rapidly growing brain appears to be more vulnerable to severe postpartum deprivation than the body as a whole. The effect is not simply a consequence of a shift of the normal growth curve to the right by 1 day, because a 1 day difference in age at 8 weeks would amount to a difference of only about 0.05 g body weight and 0.2 mg brain weight in this strain. [14]

Effects of whole-litter deprivation were clearly more severe than deprivation of one or two pups, as shown by high pup mortality and lower weight gain in the days following return to the mother. This is to be expected because the mothers, being deprived of their pups for 24 hr, were slow to resume lactation and normal maternal care. The greater severity in the short term was in several cases offset to a large extent by reduced litter size which promoted more rapid growth. Because mortality occurred in only certain litters, between-litter differences were increased, which greatly reduced statistical power and necessitated statistical correction for litter size. All things considered, the within-litter design has many advantages for examining long-term consequences of early deprivation. Even with this method there may be problems of interpretation, such as differential treatment of deprived pups by their mothers,[5] but these in no way alter conclusions of the present studies.

The corpus callosum is also reduced in size by brief postpartum deprivation to an extent that can double the frequency of mice judged abnormal. The effect is not merely a result of reduced whole brain size, because in the

BALB/c strain the two measures are very weakly correlated, even among animals in the normal range of CC size.[14] There are two plausible reasons for reduced corpus callosum size following postpartum deprivation. The treatment may reduce the number of axons which traverse the hemispheres during the rapid CC growth phase after birth,[17] or it may reduce subsequent myelination by a small amount.[9,19]

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