Prenatal protein malnutrition impairs visual discrimination learning in adult rats

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Developing rats were either malnourished or adequately nourished during the prenatal period by feeding their dams diets containing low (6% casein) or adequate (25% casein) amounts of protein for 5 weeks prior to mating and throughout pregnancy. All pups received adequate nutrition from the day of birth onwards. Male offspring were tested on the Lashley jump stand in three visual discrimination problems, beginning at 91 days of age. The previously malnourished rats made significantly more errors overall and took significantly longer to learn the three discrimination problems than did the controls. This was due primarily to inferior performance on a vertical-versus horizontal-stripe discrimination task. No significant difference between the two groups was found in brightness discrimination or in the discrimination of a square and a circle. Our results are consistent with the view that malnutrition and its associated stresses during prenatal life significantly disrupt later performance of certain complex visual learning tests.

Gestation has been implicated as the most vulnerable period for the effects of undernutrition and its associated stresses on later learning (Smart, 1977, 1985), especially pattern discrimination (Smart & Tonkiss, 1985) in the rat. There have been surprisingly few studies of visual discrimination learning in rats malnourished *solely* during the prenatal period. Galler (1981) conducted visual discrimination experiments on hooded rats provided with an adequate diet after 17–20 generations of chronic protein malnutrition. Such animals fostered from birth by well-nourished dams displayed inferior performance on a brightness discrimination task and on a horizontal- versus vertical-stripe discrimination task when tested at maturity. However, it is unclear whether these findings arose as a direct result of the gestational malnutrition or whether they might be attributable to genetic drift of the intergenerationally malnourished rat colony relative to that of the controls. That is, the two groups of rats may have become genetically dissimilar, owing to the natural selection process occurring as a result of surviving the nutritional insult. Recently, Jaiswal, Upadhyay, and Bhattacharya (1989) have reported impaired brightness discrimination in the offspring of dams fed restricted food rations during pregnancy. Pattern discrimination was not studied.

Here we report on the effect of prenatal protein malnutrition on brightness and pattern discrimination learn-

ing in the adult rat. It was found that overall performance on the tasks was significantly impaired.

METHOD

Nutritional Treatment

Five weeks prior to mating, female Sprague-Dawley VAF plus (viral and antibody-free) rats were obtained (Charles River Laboratories, Kingston, MA) as virgins and allowed ad-lib access to one of two isocaloric diets (Teklad, Madison, WI). The diets were formulated to be of adequate protein (25% casein) or low protein (6% casein) content (Galler & Tonkiss, 1991). Male rats obtained from the same source were acclimated to the experimental diets of the females for 1 week prior to mating. This procedure served to prevent any untoward effects that might have occurred if the males had encountered an unfamiliar diet when subsequently housed with the females. The animals were mated over a 10-day period. Following parturition, all litters were culled to 8 pups of 4 males and 4 females each, and they were fostered by well-nourished dams (maintained on the 25% casein diet) that had given birth no more than 36 h previously. Pups born to dams fed the 6% casein diet and cross-fostered by dams on the 25% casein diet were designated 6–25 (prenatally malnourished). Pups born to dams fed the 25% casein diet and fostered by mothers on the same diet were designated 25–25 (controls). The pups were weighed daily, starting at Day 5, until they were weaned at 25 days. Following weaning, both groups were maintained on Purina rat chow (Formula 5001). The rats had ad-lib access to food and water at all times. (For a more detailed description of the nutritional, breeding, and cross-fostering procedures, see Tonkiss & Galler, 1990.)

Laboratory Conditions

The animals were housed 2 per cage in polycarbonate microisolator cages (26 × 47 × 21 cm; Lab Products, Inc., Maywood, NJ). The temperature in the room was maintained at 73° ± 3°F. All animals were kept on a reverse 12:12-h white:red light cycle (white light on at 2000 h; red light on at 0800 h) to accommodate testing during the waking period of the rat. Behavioral testing took place in white light during the red light phase of the cycle. The animals

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were given a period of at least 15 min to adapt to white light in the test room prior to each training or test session.

Behavioral Testing

Subjects. Beginning at 91 days of age, 10 prenatally malnourished and 10 control males from different litters were selected for testing. Each rat was handled for an average of 3 min daily for 6 days prior to testing.

Apparatus. The jumping stand has been described previously (Galler & Manes, 1980) and is based on that of Lashley (1930). Briefly, the apparatus consisted of a black vertical backboard (85 × 92 cm) constructed of Plexiglas. Two circular apertures (13.4 cm in diameter, 4.4 cm apart) cut into the backboard accommodated hinged doors that could be pushed aside to allow access to a landing platform (75 × 12.5 cm) attached to the rear of the apparatus. A 13 × 14.5 cm movable platform was positioned at a height of 55 cm above the table top, in front of the doors. The distance from the platform to the doors was 14 cm during testing.

Training phase. The rats were trained, over a 7-day period, to jump through each of the apertures to the landing platform. Ten training trials were given per day with only one door clearly open; the open door appeared equally often on the left or on the right. During this time, the opening was gradually decreased in order to simulate testing conditions, and the distance to be jumped was increased from 6 to 14 cm.

Test phase. The testing procedure required the animals to discriminate between different stimuli on the two doors by jumping through the correct (unlocked) one in response to the visual cues presented. Stimulus cards were attached in front of each door: one S+, one S-. The sides for the S+ and S- on each trial were determined quasirandomly, using Gellermann's series (Gellermann, 1933). The door chosen for the S- was firmly locked from behind. The door chosen to be the S+ was shut, but left unlocked so that the rat could jump through to the landing platform behind. The rat was given 30 sec to jump spontaneously. If the rat had not jumped within this time, a single nudge (i.e., a gentle push with a rubber paddle) was administered to the rump of the animal in the direction of the backboard. This was often sufficient to encourage the rat to jump, but if the rat failed to do so within a further 15 sec, the paddle was used to gently push the rat off the platform, again in the direction of the backboard. If the rat chose correctly, it remained on the landing platform for approximately 30 sec prior to the start of the next trial. If the rat chose incorrectly, it fell a distance of 55 cm to a foam rubber surface. No other reinforcement was given.

The animals were given three discrimination tests: black versus white (B/W; W = S+); horizontal versus vertical stripes (H/V; V = S+); and circle versus square (C/S; S = S+). The discriminations were introduced in order of increasing difficulty (Lashley, 1930). The rats were given 10 trials/day for a maximum of 10 consecutive days for each discrimination task. They were considered to have learned a discrimination problem when they had achieved 90% correct responses or better on any 1 day, and they were transferred to the next discrimination task on the following day. If the animal did not reach this criterion within 10 days, it was considered to have failed the discrimination task and was given the maximum score of 10 days.

Statistical Analysis

Body weight differences between the two groups at birth and at 91 days of age were analyzed with Student's *t* tests (a one-tailed test was applied). Performance on each of the visual discrimination tests was recorded in terms of the number of days to reach criterion, the total number of errors to criterion, and the latency to jump from the platform. One-way analyses of variance (ANOVAs) were used to compare the two nutritional groups on the total scores for the three discrimination tasks and on each of

the specific discrimination tasks. An analysis of covariance (ANCOVA) was applied to control for body weight differences. All analyses were carried out with the BMDP-2V statistics program (Version 1987), which features a test for skewness.

RESULTS

One rat from the 6-25 group was removed from the study because of evidence of postnatal growth difficulties, and 1 25-25 animal was excluded because it never acquired the jumping response. Therefore, all data analyses were performed on the remaining 18 animals, with 9 rats per group. There was no obvious difference between the two groups with respect to training.

Body Weights

The birth weight of male pups in the 6-25 group ($M \pm SEM = 5.03 \pm 0.18$ g) was significantly less than that of the males in the 25-25 group [5.92 ± 0.19 g; $t(16) = 3.43$, $p < .01$]. At 91 days of age, the weights of the 6-25 males (490.56 ± 15.59 g) and the 25-25 males (535.00 ± 14.71 g) continued to be significantly different [$t(16) = 2.07$, $p < .05$].

Visual Discrimination

All animals but 1 successfully learned the three visual discrimination problems; the exception was 1 rat in the 25-25 group, which failed to reach criterion on the C/S discrimination test.

Overall Performance

The number of days to criterion was summed for the three discrimination tasks to give a total days-to-criterion score. The ANOVA showed that 6-25 animals required significantly more days to reach criterion than did the 25-25 rats [$F(1,16) = 15.88$, $p < .01$]. (See Figure 1.) The ANOVA of total errors to criterion yielded similar

Figure 1. Mean days to criterion ($\pm SEM$) of 91-day-old prenatally protein malnourished (6-25 group) and well-nourished (25-25 group) rats in learning three visual discrimination problems. Total = sum of days to criterion for all three tasks. B/W = black versus white discrimination. H/V = horizontal- versus vertical-stripe discrimination. C/S = circle versus square discrimination. ** $p < .01$ (ANOVA).

results; the 6-25 animals made significantly more errors ($M \text{ errors} \pm SEM = 46.56 \pm 4.09$) than did the controls [$M \pm SEM = 29.78 \pm 3.29$; $F(1,16) = 10.22, p < .01$]. To control for weight differences at the time of testing, the data were also analyzed with ANCOVAs. Both days to criterion [$F(1,15) = 9.78, p < .01$] and errors to criterion [$F(1,15) = 4.98, p < .05$] showed significant main effects of nutrition. These results indicate that body weight was not a significant factor in visual discrimination performance. Finally, it should be noted that there were no significant group differences in latency to leave the platform or in the proportion of rats showing fixed response patterns.

Specific Task Performance

Black/white. Although the 6-25 rats appeared to require more days to reach criterion (Figure 1) and committed more errors than the 25-25 rats did, none of these differences achieved statistical significance.

Horizontal/vertical stripes. This test provided the largest difference between the nutritional groups. The 6-25 rats required a significantly greater number of days to reach criterion than did the controls [$F(1,16) = 9.89, p < .01$; see Figure 1]—a difference that continued to be significant even when controlling for weight with an ANCOVA [$F(1,15) = 6.31, p < .05$]. When total errors to criterion were analyzed, the ANOVA showed a trend for the 6-25 animals to make more errors ($M \pm SEM = 19.00 \pm 3.41$) than the controls animals [$M \pm SEM = 12.00 \pm 1.26$; $F(1,16) = 3.71, p = .07$]. However, this difference no longer approached significance in the ANCOVA.

Circle/square. Although the 6-25 animals appeared to require more days to reach criterion (Figure 1) and committed more errors than did the 25-25 rats, statistical comparisons failed to achieve significance.

DISCUSSION

The present results indicate that protein malnutrition experienced solely during the prenatal period impairs later visual discrimination performance. The effect emerged as a significant increase in total errors committed and number of days to reach criterion on the three discrimination problems, with the deficit being particularly striking on the horizontal- versus vertical-stripe discrimination problem. These findings are important, because they are the first demonstration of an exclusively prenatal impact of malnutrition on adult visual discrimination performance in a paradigm without food as a reinforcer for task performance (see below). As such, these data may have significant implications for other visually guided behaviors, such as spatial navigation, that are currently being studied in this laboratory.

There was no significant difference between the performances of the two groups on the first, and simplest, of the three problems. It has been suggested that in postnatally malnourished rats, deficits in performance on the

first of a battery of discrimination tests reflect excessive emotional response to novel situations (Fleischer & Turkewitz, 1979; Galler, Fleischer, Turkewitz, & Manes, 1980) or an inability to ignore irrelevant position cues (Castro & Rudy, 1989). Although a trend toward poorer performance was noted in the prenatally malnourished rats, the absence of a significant effect suggests that high emotionality and/or the use of inflexible position strategies are not consequential to prenatal nutritional stress. Contrary to these findings, Jaiswal et al. (1989) found significantly impaired performance on a food-rewarded black versus white discrimination task in the offspring of rats whose mothers had been protein-calorie malnourished during pregnancy. Although there are a number of differences between that study and the present one, the most significant may be the use of food motivation in the Jaiswal et al. study. We have documented heightened response to food reward (Tonkiss, Shukitt-Hale, Formica, Rocco, & Galler, 1990) in prenatally malnourished adult rats. Thus, differential motivation for the reinforcer may have been implicated in the outcome of Jaiswal et al.'s (1989) discrimination experiment.

Impaired learning performance of the prenatally malnourished rats on the horizontal- versus vertical-stripe discrimination task is a finding strengthened by the fact that it emerged after the rats had mastered the generalities of the task. Castro and Rudy (1989) have suggested that the order of presentation of discrimination tests can have a significant influence on behavioral outcome. In their study, performance differences on a horizontal- versus vertical-stripe discrimination task in postnatally malnourished rats could be eliminated by prior experience of a black versus white discrimination task. Similarly, performance differences on a black versus white discrimination problem could be eliminated by previous experience of a horizontal- versus vertical-stripe discrimination problem. Clearly, this was not the case in the present study. However, prior experience of a horizontal- versus vertical-stripe test cannot be ruled out as a factor contributing to the absence of a significant deficit on the most difficult discrimination test (circle vs. square). Examination of performance in naive rats would be needed to determine the independent effects of each discrimination problem. Note, however, that two studies of the impact of postnatal malnutrition on discrimination of a circle and square without prior experience of other discrimination problems (Rogers, Tonkiss, & Smart, 1986; Smart, Billing, Duggan, & Massey, 1989) both indicated equality of learning performance in previously malnourished and control animals.

The mechanism underlying impaired discrimination of stripe orientation is worth considering. Though no data have been collected on the visual cortex of rats malnourished solely during the prenatal period, deficits in dendritic arborization of layer V pyramidal cells and increased spine densities in regions close to the cell body have been observed in chronically protein-malnourished 90-day-old rats (Diaz-Cintra, Cintra, Ortega, Kemper, &

Morgane, 1990). These rats were born to dams fed a mildly protein-restricted diet (8% casein) 5 weeks prior to mating and throughout pregnancy and lactation. At weaning, the offspring were placed on the same restricted diet. Recent observations, also from our group, of other brain regions (Diaz-Cintra et al., in press) suggest that the moderate prenatal protein malnutrition used in the present investigation (6% casein diet) may have even more pronounced effects on neuroanatomical parameters than chronic mild protein malnutrition (8% casein diet; Cintra, Diaz-Cintra, Galvan, Kemper, & Morgane, 1990). Thus, an effect on visual cortex seems likely. Deficient pattern discrimination but spared brightness discrimination has been found in rats with developmental impairment in the caudal regions of the cortex (including visual projection areas) caused by treatment of their dams with a teratogenic agent (methylazoxymethanol acetate) in the final week of pregnancy (Pereira, Legg, Steele-Russell, & Glickstein, 1985), and in adult animals with lesions of the visual cortex (de Vos Korthals & van Hof, 1981; Lavond & Dewberry, 1980). Clearly, there is a need for multidisciplinary studies in which anatomical measures of the visual cortex are related with discrimination performance in prenatally malnourished rats.

Finally, a consideration of the specificity of the prenatal treatment is warranted. Since prenatal malnutrition results in metabolic (Zartarian, Galler, & Munro, 1980) and behavioral changes in the dam (Galler, Zartarian, Neel, & Munro, 1980), it is not possible to separate the purely nutritional effects on the developing offspring from the effects of stress on the dam during this period. However, fostering of the malnourished pups by well-nourished dams from birth results in only minimal changes in mother-infant interaction (Galler & Tonkiss, 1991), enabling the conclusion that the postnatal environment of the young animals is relatively unaffected by malnutrition and other associated factors experienced during gestation.

REFERENCES

- CASTRO, C. A., & RUDY, J. W. (1989). Early-life malnutrition impairs the performance of both young and adult rats on visual discrimination tasks. *Developmental Psychobiology*, **22**, 15-28.
- CINTRIA, L., DIAZ-CINTRIA, S., GALVAN, A., KEMPER, T., & MORGANE, P. J. (1990). Effects of protein undernutrition on the dentate gyrus in rats of three age groups. *Brain Research*, **532**, 271-277.
- DE VOS KORTHALS, W. H., & VAN HOF, M. W. (1981). Pattern discrimination after unilateral and bilateral occipital lobe ablation in the rabbit. *Behavioural Brain Research*, **2**, 219-222.
- DIAZ-CINTRIA, S., CINTRA, L., GALVAN, A., AGUILAR, A., KEMPER, T., & MORGANE, P. J. (in press). Effects of prenatal protein deprivation on the postnatal development of granule cells in the fascia dentata. *Journal of Comparative Neurology*.
- DIAZ-CINTRIA, S., CINTRA, L., ORTEGA, A., KEMPER, T., & MORGANE, P. J. (1990). Effects of protein deprivation on pyramidal cells of the visual cortex in rats of three age groups. *Journal of Comparative Neurology*, **292**, 117-126.
- FLEISCHER, S. F., & TURKEWITZ, G. (1979). Effect of neonatal stunting on development of rats: Large litter rearing. *Developmental Psychobiology*, **12**, 137-149.
- GALLER, J. R. (1981). Visual discrimination in rats: The effects of rehabilitation following intergenerational malnutrition. *Developmental Psychobiology*, **14**, 229-236.
- GALLER, J. R., FLEISCHER, S. F., TURKEWITZ, G., & MANES, M. (1980). Varying deficits in visual discrimination performance associated with different forms of malnutrition in rats. *Journal of Nutrition*, **110**, 231-240.
- GALLER, J. R., & MANES, M. (1980). Gender differences in visual discrimination by rats in response to malnutrition of varying durations. *Developmental Psychobiology*, **13**, 409-416.
- GALLER, J. R., & TONKISS, J. (1991). Prenatal protein malnutrition and maternal behavior in Sprague-Dawley rats. *Journal of Nutrition*, **121**, 762-769.
- GALLER, J. R., ZARTARIAN, G. N., NEEL, A. L., & MUNRO, H. N. (1980). Marginal protein deficiency in pregnant rats: II. Impaired behavior during pregnancy. *Journal of Nutrition*, **110**, 1298-1302.
- GELLMERMANN, L. W. (1933). Chance orders of alternating stimuli in visual discrimination experiments. *Journal of General Psychology*, **42**, 206-208.
- JAISWAL, A. K., UPADHYAY, S. N., & BHATTACHARYA, S. K. (1989). Effect of piracetam, a nootropic agent, on discrimination learning deficits induced by parental undernutrition and environmental impoverishment in young rats. *Indian Journal of Experimental Biology*, **27**, 269-273.
- LASHLEY, K. S. (1930). The mechanism of vision: I. A method for rapid analysis of pattern-vision in the rat. *Journal of General Psychology*, **37**, 453-460.
- LAVOND, D. G., & DEWBERRY, R. G. (1980). Visual form perception is a function of the visual cortex: II. The rotated horizontal-vertical and oblique stripe problems. *Physiological Psychology*, **8**, 1-8.
- PEREIRA, S. C., LEGG, C. R., STEELE-RUSSELL, I., & GLICKSTEIN, M. (1985). Functional deficits and anatomical sparing after prenatal brain damage in the rat. In B. E. Will, P. Schmitt, & J. C. Dalrymple-Alford (Eds.), *Brain plasticity, learning, and memory* (pp. 321-330). New York: Plenum.
- ROGERS, P. J., TONKISS, J., & SMART, J. L. (1986). Incidental learning is impaired during early-life undernutrition. *Developmental Psychobiology*, **19**, 113-124.
- SMART, J. L. (1977). Early life malnutrition and later learning ability: A critical analysis. In A. Oliverio (Ed.), *Genetics, environment and intelligence* (pp. 215-235). Amsterdam: Elsevier/North-Holland.
- SMART, J. L. (1985). Undernutrition, learning and memory: Review of experimental studies. In T. G. Taylor & N. K. Jenkins (Eds.), *Proceedings of the 12th Congress on Nutrition*. London: Libbey.
- SMART, J. L., BILLING, A. E., DUGGAN, J. P., & MASSEY, R. F. (1989). Effects of early life undernutrition in artificially-reared rats: 3. Further studies of growth and behaviour. *Physiology & Behavior*, **45**, 1153-1160.
- SMART, J. L., & TONKISS, J. (1985). Visual discrimination learning in rats following early life undernutrition: Recent findings and review. In B. E. Will, P. Schmitt, & J. C. Dalrymple-Alford (Eds.), *Brain plasticity, learning, and memory* (pp. 331-341). New York: Plenum.
- TONKISS, J., & GALLER, J. R. (1990). Prenatal protein malnutrition and working memory performance in adult rats. *Behavioural Brain Research*, **40**, 95-107.
- TONKISS, J., SHUKITT-HALE, B., FORMICA, R. N., ROCCO, F. J., & GALLER, J. R. (1990). Prenatal protein malnutrition alters response to reward in adult rats. *Physiology & Behavior*, **48**, 675-680.
- ZARTARIAN, G. N., GALLER, J. R., & MUNRO, H. N. (1980). Marginal protein deficiency in pregnant rats: I. Changes in maternal body composition. *Journal of Nutrition*, **110**, 1291-1297.

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