Reversal of spatial discrimination learning in a water maze by previously undernourished rats

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The development of the hippocampus in rats may be vulnerable to undernutrition during the fetal and suckling periods. Hence the behavioral effects of early growth restriction may resemble those of hippocampal lesions. This suggestion was investigated by testing previously undernourished rats for reversal learning, an ability badly affected in hippocampectomized rats. Developing rats were undernourished by feeding their mothers a restricted quantity of a good quality diet during pregnancy and lactation. All rats were fed ad lib from weaning. Reversal of spatial discrimination learning was tested in adult animals using a water T-maze. Previously undernourished rats learned the initial spatial discrimination more quickly than controls. However, there was no effect of early treatment on serial reversal learning in Experiment I or on performance of a single reversal after prolonged initial training in Experiment II.

Nutritional deprivation during brain growth and development has been shown to produce a number of alterations of brain structure and biochemistry. It has been difficult, however, to devise investigations directly relating the disordered brain to altered behavior (Dobbing & Smart, 1974). An exception to this generalization is the selective effect of undernutrition at the time of the brain growth spurt on the cerebellum, which is paralleled in rats by lasting effects on motor coordination (Lynch, Smart, & Dobbing, 1975). This special vulnerability of the cerebellum probably relates to both the speed and timing of its growth. It grows very much faster over a shorter period than the rest of the brain. In a similar way, the hippocampus may also be vulnerable, since, with the cerebellum, it is one of the few brain regions in which neuronal multiplication occurs post-natally in the rat (Bayer & Altman, 1974). Its development may well proceed abnormally in underfed young. Hence there may be similarities in the behavior of previously undernourished rats and those with hippocampal lesions. [It is appreciated, however, that early undernutrition does not produce brain lesions (Dobbing & Smart, 1973).]

Reversal learning was chosen for study because it is known to be severely impaired in hippocampectomized animals (see Altman, Brunner, & Bayer, 1973; Douglas, 1967; Kimble, 1968). Correspondences between the effects of the two treatments on other behaviors are discussed below (p. 315).

Interpretation of the effects of early undernutrition on learning ability is often clouded by motivational factors (Dobbing & Smart, 1973). For instance, previously undernourished rats are more reactive to electric shock (Levitsky & Barnes, 1970) and are more responsive in both food-getting situations (reviewed by Bronfenbrenner, 1968; Smart, Dobbing, Adlard, Lynch & Sands, 1973) and water-getting situations (Smart & Dobbing, Note 1). Escape from water was used as motivation for learning in the present investigation in an attempt to avoid these difficulties.

There has already been some study of reversal of spatial discrimination learning in water mazes during or after periods of nutritional deprivation. Barnes, Cunnold, Zimmermann, Simmons, MacLeod, and Krook (1966) found that young male rats, still in a state of malnutrition, showed impaired reversal of position learning in a Y-maze. However, older rats rehabilitated from undernutrition in early life showed no deficit in reversal learning (Cravens, 1974). Barnes' rats performed one reversal task and Cravens' rats three. An attempt was made in the present study to investigate in greater depth the reversal performance of previously undernourished rats. In Experiment I, *serial* learning of spatial discrimination was investigated. Reversal performance of rats *overtrained* in the original discrimination was studied in Experiment II.

METHODS

Subjects

The rats were of the black-and-white hooded Lister strain. Conditions of rearing, mating routine, and composition of diet

have been described in detail elsewhere (Smart & Dobbing, 1971; Smart et al., 1973). All mother rats had already borne and reared one litter. Control mothers had free access to a good-quality diet at all times; undernourished mothers were fed a restricted quantity of the same diet daily, which was about half that taken by control mothers. This amount increased from 10 g per day throughout pregnancy to 15 g during the first week of lactation, 20 g in the second week, and 25 g from Day 15 to Day 25 when the young were weaned. All rats were fed ad lib from weaning. Water was always freely available to all rats.

Litters were reduced to eight young on the day of birth, where possible to five males and three females. Only males were kept beyond weaning, and these were housed two or three per cage in littermate groups until shortly before testing. Rats were weighed at birth, weaning, and 6, 9, 12, and 15 weeks of age. The illumination of the animal rooms was on a 12-h white light/12-h red light cycle, switching from white light to red light at 12.00 h. Behavior was tested between 09.00 and 12.00 h.

Apparatus

The T-shaped water maze described by Smart and Adlard (1974) was used. It was filled to a depth of 230 mm with water at a temperature of 22°C.

Procedure

Experiment 1. Seventeen control (C) and 11 previously undernourished (PU) male rats were used. These were drawn from seven C and five PU litters. The rats were housed singly throughout the period of testing, which began at about 14 weeks.

Serial reversal learning of spatial discrimination was tested exactly as described by Smart and Adlard (1974) for guinea pigs. Briefly, rats were tested on 20 consecutive days, five trials a day, except on Day 1 and on the first day after each criterion of reversal learning had been met, when six trials were given. The criterion was five out of five consecutive trials correct on any 1 day. Rats were allowed to correct wrong turnings without being removed from the maze, and errors were scored when they entered the wrong arm or retraced their path into the start arm.

Experiment 2. Sixteen C rats from seven source litters and 13 PU rats from six litters were tested. All had been tested for their aversion threshold to electric shock at about 15 weeks of age (Smart, Whatson, & Dobbing, 1975). Tests in the water maze began at 17 weeks. The rats were caged singly throughout the whole test period.

Alternate rats were assigned for training to the left or to the right side. For any individual rat, the position of the escape platform remained the same on Days 1 to 5, irrespective of performance. On Day 6, the position of the escape platform was reversed and the situation remained thus on Day 7. Each rat was given 10 trials a day throughout. A "correction" procedure was again adopted.

RESULTS

Body Weight

Body weights of the present rats are tabulated elsewhere (Smart, Whatson & Dobbing, 1975). There were highly significant differences in body weight between C and PU rats at all ages from birth to 15 weeks.

Experiment 1

Both groups showed the pattern of errors typical of serial reversal learning by rats (Mackintosh, 1969). Errors increased from the first to the second reversal, but declined steadily thereafter.

Table 1
Performance of Control (C) and Previously Undernourished (PU) Rats in the Water Maze in Experiment I (means ±S.E.)

Measure	C	PU
Number of rats	17	11
Day first criterion met	2.6 ± .2	2.5 ± .3
Total reversals	8.2 ± .5	8.9 ± .9
Total errors	37.9 ± 1.4	43.2 ± 2.9

There were no significant differences between C and PU rats on any measure of performance (Table 1).

Experiment 2

PU rats learned the initial spatial discrimination more efficiently than C rats. They attained earlier a criterion of 10 out of 10 runs correct on any 1 day (p < .005, t test). One PU rat fortuitously attained this criterion on Day 1 (Figure 1). Even with this result omitted from the analysis, the significance of the difference remains at the same level. PU rats also made fewer errors before attaining the criterion (mean ± S.E.: 4.3 ± 0.9 compared with 8.4 ± 1.1, p < .01, t test). With the aberrant rat excluded, the significance level is p < .02.

By Days 4 and 5, performance was near perfect in both groups (Figure 2). There were no differences between groups in reversal learning on Days 6 and 7.

DISCUSSION

The superior initial learning by PU rats in Experiment 2 is puzzling, especially as there was no such
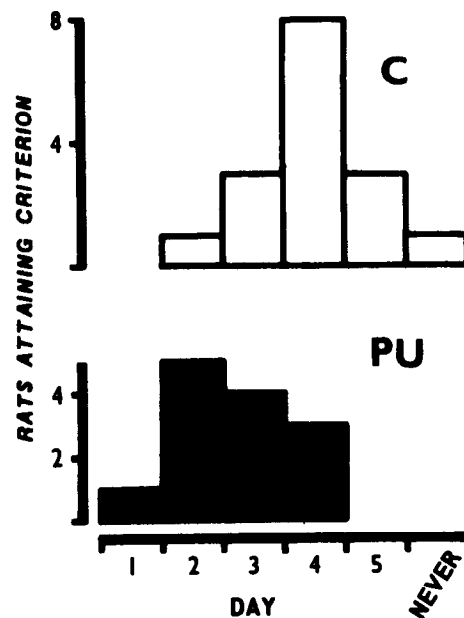

Figure 1. Frequency distributions of the number of rats which first achieved the criterion of 10 out of 10 runs correct on Days 1 to 5 of Experiment 2. C = control rats; PU = previously undernourished rats.
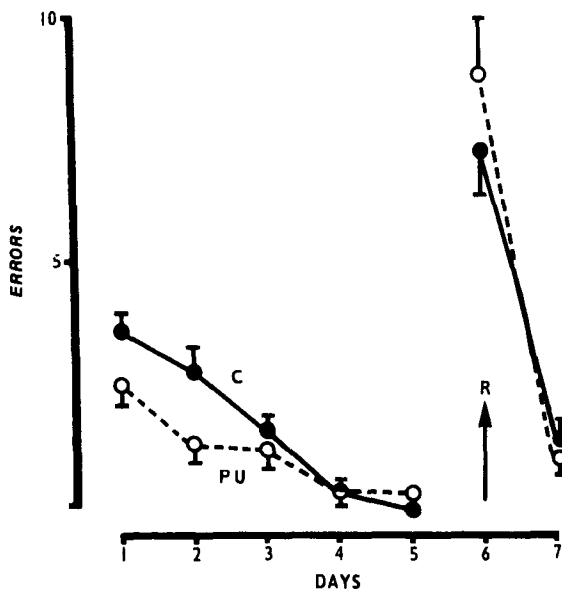
Figure 2. Mean (±S.E.) errors per day by control (C) and previously undernourished (PU) rats in Experiment 2. Reversal (R) of the initial spatial discrimination was tested on Days 6 and 7.

difference in Experiment 1. One procedural difference between the two experiments which may be relevant is that side preferences were established in Experiment I and initial training was to the non-preferred side, whereas in Experiment II rats were assigned alternately for training to the left or right sides. A higher proportion of PU rats may, by chance, have been assigned to their preferred side, thus giving them an initial advantage. However, this is not borne out by the results of their first trial in the water maze. Five out of 13 PU rats made correct choices, as did 6 out of 16 C rats. The result remains an enigma. It is perhaps noteworthy that Howard and Granoff (1968) found PU mice to be better than controls at a delayed-response visual discrimination in a water Y-maze.

One way of accounting for the PU rats' faster learning in Experiment II is to postulate differences in motivation. Being placed in water may be more unpleasant for them. Such rats are leaner than well-nourished controls; that is, they have smaller fat deposits for their size (Smart, Adlard, & Dobbing, 1974). Hence, the PU rats may have been denser and sunk lower in the water than the controls, and they may have been less well insulated against the cool water. Moreover, it has been suggested that they are more responsive to noxious stimulation in general (Levitsky & Barnes, 1970).

The hypothesis that rats growth-retarded during gestation and the suckling period might resemble rats with hippocampal lesions in their behavior is not supported by the results. There were no differences in reversal learning in either experiment. The correspondence of the effects of early undernutrition with

those of hippocampal lesions is good for some behaviors but not for others. (For information on hippocampal lesions see reviews by Altman et al., 1973; Douglas, 1967; Kimble, 1968). Both hippocampectomized and PU rats show slow extinction of learned responses (Fraňková & Barnes, 1968; Simonson & Chow, 1970), and both barpress for food at a high rate on operant conditioning schedules of the variable interval type (Smart et al., 1973). However, the two treatments have diametrically opposed effects on performance in passive avoidance situations. Hippocampectomized rats perform poorly, whereas PU rats show enhanced passive avoidance (Levitsky & Barnes, 1970; Smart et al., 1973). Likewise, there are differences in behavior on open-field tests. Rats with hippocampal lesions are hyperactive; PU rats are, if anything, less active than controls in such short duration tests (see Smart, 1974).

The level of comparison above may be too crude. A more appropriate analogy may be lesioning of developing animals rather than adults, and specifically lesioning of regions theoretically vulnerable to early undernutrition. The dentate gyrus of the hippocampus, 85% of whose granule cells are formed postnatally, is such a region (Bayer & Altman, 1974). However, rats deprived by X-irradiation during the suckling period of most of the granule cells of the dentate gyrus were found to behave in adulthood very much like rats subjected to hippocampal lesions as adults (Bayer, Brunner, Hine, & Altman, 1973).

Hence, there is no simple involvement of the hippocampus or even the dentate gyrus in producing the behavioral effects of early undernutrition. Even though there may be some selective effect on the hippocampus (and this has yet to be conclusively demonstrated), it is not to the exclusion of effects on other brain regions. It is probably safest to propose that the final behavioral outcome of early undernutrition is the product of the subtle interaction of effects on several brain regions, some of which may contribute more than others, and possibly of effects on endocrine systems (see Adlard & Smart, 1972).

REFERENCE NOTE

1. J. L. Smart & J. Dobbing, *Increased thirst and hunger in adult rats undernourished as infants: An alternative explanation.* In preparation.

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