Facilitative Effects of Maternal Environmental Enrichment on Maze Learning in Rat Offspring

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KIYONO, S., M. L. SEO, M. SHIBAGAKI AND M. INOUYE. Facilitative effects of maternal environmental enrichment on maze learning in rat offspring. PHYSIOL BEHAV 34(3) 431-435, 1985.—Pregnant rats were differentially reared in enriched (EC), impoverished (IC), and standard colony conditions (SC) through the pregnancy. Half of the male offspring were reared by their biological mothers and the remaining half were reared by foster-mothers. After weaning male offspring were tested in the Hebb-Williams maze apparatus. The effect of environment was significant for the total error scores; the EC group had less errors than the IC group. In a second experiment all male offspring (EC, SC and IC) were reared by foster-mothers. The effect of environment was significant for initial, repetitive, and total error scores. Further analysis revealed that the EC-SC and EC-IC differences were significant, whereas the IC-SC difference was not. Thus, the results obtained were the first to reveal that maternal environmental enrichment during pregnancy can exert a facilitatory influence on the postnatal maze learning abilities of the offspring.

Prenatal environmental enrichment Maze learning

REARING animals in environmentally enriched conditions has been shown to affect brain morphology and chemistry as well as behavioral performance in numerous experiments (see [21, 26, 27] for reviews). Environmental enrichment has been further utilized as a tool ('environmental therapy') to alleviate the disadvantaged learning abilities such as experimental cretinism [5] and microencephaly due to prenatal X-irradiation [13, 23, 24]. The effects, however, have almost exclusively been studied in animals differentially reared postnatally, mainly after weaning. In a previous study we reported that facilitative effects of prenatal environmentally enriched conditions (EC) could be observed as a postnatal decrease of initial error scores in the Hebb-Williams maze learning as compared with the isolated, environmentally impoverished (IC) group [14]. However, it should be noticed that the results remained merely suggestive, since the sample size was too small to make any definitive conclusion. Second, the question remained whether it was due to EC or IC effect, since no standard colony condition (SC) group was used as a control reference. Thirdly, the effect could not be attributed only to prenatal EC, since all animals were reared by their real mothers and the possible postnatal mothering effect could not be neglected. Accordingly, in the present report, prenatal effects on postnatal maze learning ability were studied in rats whose mothers had been placed in different environmental conditions during pregnancy, using an additional SC group and a fostering technique [12].

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TABLE 1

<table>
<thead>
<tr>
<th>Effect of</th>
<th>Biol</th>
<th>Fost</th>
<th>Fost Envir</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC</td>
<td>97.4 ± 6.8 g</td>
<td>98.2 ± 5.5 g</td>
<td>F=1.34</td>
<td>F=10.83*</td>
</tr>
<tr>
<td>SC</td>
<td>93.6 ± 7.7 g</td>
<td>91.3 ± 5.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC</td>
<td>91.8 ± 6.8 g</td>
<td>88.7 ± 11.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± S.D., Fost: fostering; Envir: environment.
*p<0.05, df=2, 125.

TABLE 2

<table>
<thead>
<tr>
<th>Effect of</th>
<th>Biol</th>
<th>Fost</th>
<th>Fost Envir</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial, Repetitive, and Total Errors Per Trial, Summed for the 12 Problems</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>14.7 ± 0.7</td>
<td>15.2 ± 0.7</td>
<td>16.4 ± 0.5</td>
<td>15.9 ± 0.6</td>
</tr>
<tr>
<td>SC</td>
<td>2.8 ± 0.3</td>
<td>2.6 ± 0.3</td>
<td>2.6 ± 0.2</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>IC</td>
<td>17.5 ± 0.9</td>
<td>17.7 ± 0.8</td>
<td>19.0 ± 0.7</td>
<td>18.8 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>F=2.69*</td>
<td>F=0.46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean ± S.E.M., *p<0.10, ?p<0.05.
Df’s are 2, 125 in all cases.

On day 20 of pregnancy the females were removed from their particular environments and placed in individual SC cages with appropriate bedding material and allowed to deliver undisturbed. Pups were nonsystematically culled to four males and four females per litter whenever possible on the next day after birth. Offspring born to five EC, five SC and six IC dams were reared by their biological mothers (B), and the remaining pups born to five EC, five SC and four IC dams were fostered (F) by the SC mothers who had delivered within 24 hr. The pups born to these foster-mothers were removed after the assignment. At 21–25 days of age pups were weaned and male offspring were used for maze tests. The subjects were, thus, 23 EC-B (prenatal EC followed by postnatal rearing by the biological mother), 19 EC-F, 23 SC-B, 23 SC-F, 21 IC-B and 21 IC-F offspring. They were coded to prevent experimental bias, and three or four litters were placed in SC cages according to rearing conditions, and deprived of water for 24 hr. From the next day on, food pellets were given ad lib, but water was not provided in the home cage. However, mashed food, 15–20 g of pellets mashed with 30–40 ml of water per rat, was given daily at the home cage after training.

Using a standard Hebb-Williams maze [19], preliminary training was then started until the total running time in 9 trials was less than 60 sec for two consecutive days. Water was used as a reward. They were then given 8 trials on one problem per day. This was continued until all 12 problems were completed. In each trial of the maze task, three kinds of errors were scored: initial, repetitive, and total errors. An initial error (IE) is the first made in a given error zone on a given trial; repetitive errors (RE) are further errors made in the same zone on a given trial. Total error (TE) is the sum of IE and RE.

The difference of means was examined using ANOVA and any further analysis necessary was made by the multiple comparison with the protracted LSD method and partly by the Student t test.

After completion of the experiment, rats were anesthetized and sacrificed. Five to eight brain samples were randomly taken from each of the six groups and coded to prevent experimental bias. Wet brain (cut at the obex) and cerebral weights (cut between the superior and inferior colliculi) were measured to 0.001 g. Thickness of the occipital cortex was measured according to Diamond et al. [6]. Using 10 occipital samples from three EC and 7 samples from three IC rats, dendritic spines of the pyramidal neurones were counted on the terminal, apical, oblique and basal dendrites according to Globus et al. [9].

Results

Litter sizes were 10.0±0.9 (mean±S.D.), 9.7±1.6 and 10.6±1.2 and male body weights at birth were 5.2±0.3 g, 5.3±0.5 g and 4.9±0.4 g for the EC, SC and IC groups, respectively. Neither difference was significant. Table 1 shows the body weights at the start of the maze test. The effects of fostering and the interaction were not significant, whereas the effect of environment was $F(2,125)=10.83$, $p<0.05$.

Table 2 shows that TE yielded a significant effect of environment, $F(2,125)=3.08$, $p<0.05$, but not of fostering. The Fostering × Environment interaction was not significant. IE and RE also yielded a similar effect of environment, but did not reach the significance level at $F(1,125)=2.69$, $p<0.10$ and $F(2,125)=2.67$, $p<0.10$, respectively. Since the effect of fostering was not significant, the B and F groups were combined and the differences in the TE scores among the 3 groups were examined. A significant difference was found only between EC and IC groups, $t(124)=2.56$, $p<0.05$. The EC-SC and IC-SC differences were not significant.
In Table 3, neither the brain weight nor the cortical thickness yielded significant effects of environment and fostering. However, it is interesting to note that the values in the SC group were situated between those in the EC and IC groups, the former being larger than the latter for both items. The number of dendritic spines also revealed no specific differences between the EC and IC groups. A second experiment was conducted to replicate and extend these findings.

EXPERIMENT II

Method

Since the second experiment was a replication and expansion of Experiment I, only the differences will be noted. Thirty-two pregnant rats were randomly assigned to the EC (n=8), SC (n=17) and IC (n=7) groups. In addition to the social grouping with toys in a large cage as in the previous experiment, EC dams were allowed to explore freely for an hour per day, three times a week, in the Hebb-Williams maze apparatus without any barrier patterns. Offspring born to six EC, seven SC and seven IC dams were reared by the SC mothers who had delivered within 24 hr from the next day after birth; in this series no litter was reared by its biological mother. Thus, the subjects for maze study were twenty male rats for each group.

In the course of the practice problem training, all rats were trained to leave the start box as soon as they were put into the box with the simultaneous quick elevation of the guillotine door. Accordingly, after completion of the preliminary training, start latencies were practically zero for all animals.

Results

No differences in the body weights were found between the 3 groups at the start of the maze test; 68.3±3.4 g, 67.5±3.5 g and 66.7±3.1 g for the EC, SC and IC groups, respectively. At the end of the test, however, body weight of the EC group (110.7±7.0 g) was significantly heavier than that of the SC group (84.9±23.0), t(18)=3.56, p<0.005. No difference was found between the SC and IC (82.9±22.3 g) groups.

Figure 1 shows the TE scores for each of the 12 problems in the 3 groups. The general patterns are quite similar to the data obtained in the previous experiment. Although problems differ in the degree of difficulty, a certain tendency could be seen in that the TE scores in EC group (inverted solid triangles) tended to be less than those of IC (solid triangles), and the SC scores (open circles) fell in between the two groups. Table 4 indicates that the prenatal environmental factor affects significantly the postnatal maze performance; effect of environment was significant for the IE, RE and TE scores. Further analysis revealed a significant difference in RE scores between the EC and SC groups,
The present results are perhaps the first to present experimental corroboration for this traditionally popular concept. A pioneering work of Ivinskis and Homewood [11] stated that exposure of the pregnant female rat during the last trimester in EC (two rats in an EC cage) had no significant effect on problem-solving behavior tested in the Hebb-Williams maze when offspring were tested 64 days later. Instead of the prenatal influence, they suggested that the beneficial enrichment effect might be mediated by the mother, who, in some way, transmits additional stimulation to the infant rat during the early postnatal stage of development.

The possible effects of postnatal maternal influences were examined in Experiment I, in which the offspring were reared either to their real mothers or foster-mothers, and in Experiment II, in which all offspring were reared by foster-mothers. Thus, the postnatal effects of maternal behavior was coordinated by the maternal one [20]. McKim et al. [15] have reported that variation in environmental complexity undergone by mother rats during pregnancy can produce definite changes in offspring behavior in the open-field test. From these findings it can be safely supposed that fetal life is closely related with the outer world, not only with the intrauterine environment, but also with the external world via the maternal body. The present results suggest that prenatal maternal enrichment has a beneficial effect on postnatal learning of the offspring, although the mechanism remains to be solved.

**ACKNOWLEDGEMENT**

The authors are indebted to Mr. T. Tachibana for his assistance in the statistical analyses.

**REFERENCES**


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**TABLE 4**

<table>
<thead>
<tr>
<th></th>
<th>EC</th>
<th>SC</th>
<th>IC</th>
<th>Effect of Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>IE</td>
<td>12.3 ± 0.5</td>
<td>13.7 ± 0.6</td>
<td>14.0 ± 0.5</td>
<td>F=2.92 df=2.57 p=0.06</td>
</tr>
<tr>
<td>RE</td>
<td>2.4 ± 0.1</td>
<td>3.1 ± 0.3</td>
<td>3.6 ± 0.2</td>
<td>6.54  2, 57 =0.003</td>
</tr>
<tr>
<td>TE</td>
<td>14.7 ± 0.6</td>
<td>16.8 ± 0.9</td>
<td>17.6 ± 0.7</td>
<td>4.36  2, 57 =0.017</td>
</tr>
</tbody>
</table>

Mean ± S.E.M.

$t(57)=2.12, p=0.036$. The EC-IC difference was also significant, $t(57)=3.60, p=0.001$, whereas the IC-SC difference was not. For the TE scores the EC-SC and EC-IC differences were significant ($t(57)=2.03, p=0.044$ and $t(57)=2.87, p=0.006$, respectively), whereas the IC-SC difference was not.


