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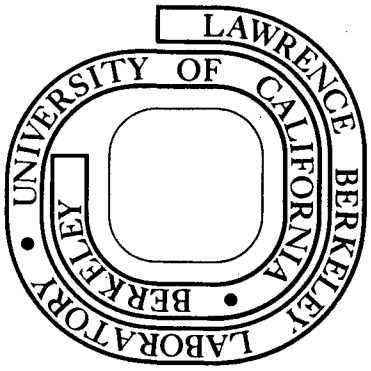

DISCLAIMER

SOCIAL GROUPING CANNOT ACCOUNT FOR CEREBRAL OR BEHAVIORAL EFFECTS OF ENRICHED ENVIRONMENTS

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SUMMARY

Several experiments were conducted to test whether mere group living can account for cerebral and behavioral differences that have been reported to develop between rodents housed in groups in enriched environments and rodents housed singly in restricted environments. Groups of 12 male rats were assigned for 30 days to several types of environment: (a) large cage with varied stimulus objects, (b) large cage without stimulus objects, (c) large cage with complex maze, pattern changed daily, (d) seminatural outdoor environment. Although the number of rats housed together was the same in these cases, and cage size was the same for conditions a - c, significantly different effects were found in weights, acetylcholinesterase, and RNA/DNA of cerebral cortex; the cerebral effects were largest in condition d and smallest in b. When 48 rats were placed in three interconnected cages, however, the presence or absence of stimulus objects did not produce differences in brain values. In another experiment, intact or brain-injured rats were tested in the Hebb-Williams maze after having spent 90 days in one of three conditions: (a) 3 per colony cage, (b) 12 in a large cage without stimulus objects, (c) 12 in a large cage with stimulus objects. Both among the intact and the brain-lesioned rats, the groups that had had access to stimulus objects performed significantly better than the others, so here too the effect of the enriched environment could not be attributed solely to the social factor.

INTRODUCTION

Although numerous studies have shown that rodents housed in groups in a complex environment develop significant differences in brain weights, brain chemistry, and brain anatomy from similar animals housed singly in a restricted environment (see reviews by Greenough[7] and Rosenzweig & Bennett[16]), the question has been raised by Welch et al.[23] whether mere group living suffices to alter the brain values without a complex inanimate environment being required. The study of Welch et al. did demonstrate differences in brain weights and nucleic acids as a result of housing rats in groups of 8-10 versus isolating them for a one-year period; these results were no surprise since it had already been shown that housing rats in groups of 3 versus isolation for an 80-day period produces significant differences in brain weights and brain enzymes.[20] But the effectiveness of social grouping did not test whether still larger effects could have been produced by combining inanimate with social stimulation; this combination of inanimate and social stimulation has characterized most of the research on "enriched" or "complex" environments. In behavioral experiments, Morgan[10] compared isolated rats with rats kept in social groups and with rats given the combination of social grouping and presence of varied stimulus objects. He reported that while both social groups were superior to the isolated animals in acquisition and reversal of complex motor skills, the social groups did not differ from each other, so he attributed the behavioral differences entirely

to the social factor. We have shown that the conclusion reached in this sort of experiment depends in part on the behavioral test employed[14]: In the Visual Reversal Test in the Krech apparatus, similar numbers of errors were made by both animals from the social groups of 3 and by those from the groups in the complex environment, and both were superior to the isolated rats; in this case, social grouping alone could account for the differences. However, in the Lashley III maze, rats that have been isolated make similar numbers of errors as rats that have lived in groups of 3, and both make significantly more errors than rats that have lived in groups of 12 in a complex environment; here the social factor could not account completely for the differences.[14]

The present paper reports experiments in which effects of housing rats in a social group of 12 are compared with effects of providing various types of inanimate stimulation to groups of the same size. Thus the present experiments vary the environment while keeping the size of the social group constant and so permit a clearer test of effects of inanimate environment than had been afforded by earlier work. Effects are measured in terms of brain weights, nucleic acids, and acetylcholinesterase, and also in terms of scores on the Hebb-Williams test of problem-solving behavior. In the case of brain measures, the combined stimulation produced significantly larger effects than did social grouping alone; in the case of the problem-solving scores, the combination of inanimate and social grouping had significant

effects in reducing errors, whereas social grouping alone did not produce significant effects.

METHODS

Subjects

Subjects were male rats of the Berkeley S_1 line bred in the Department of Psychology colony. In most experiments they were assigned to experimental conditions at about 25 days of age and were kept in conditions for about 30 days. Depending upon the number of conditions included in an experiment--3, 4, or 5--we chose litters with at least 3, 4, or 5 male animals. Runts were excluded, and, as a further restriction on variability, we took only sets of littermates in which the range of body weights within the litter did not exceed 15% at the time of assignment to conditions. The littermates were then assigned randomly to conditions so that each animal of a litter went to a different experimental condition.

Environmental conditions

Our standard enriched condition (EC), with a group of 12 animals housed in a large cage that is provided with various inanimate stimulus objects, was included as a reference condition in these experiments, and other groups of 12 rats were provided with other environments in order to compare the effects with those of EC. The standard impoverished condition (IC), with a single animal housed in a small cage, was included as another

reference condition. The various experimental conditions are described next, and Table I presents the summary of them.

In the Enriched Condition (EC) 12 rats are housed in a relatively large cage (75 cm wide x 75 cm deep x 45 cm high) which is furnished with about six varied stimulus objects from a pool of 25 objects. Several EC cages are set up adjacent to each other, and each day the animals are moved from one cage to another; after several days stimulus objects are changed in all cages so that the animals will be exposed to new objects and new combinations of objects. In this and all other conditions, food and water are available ad libitum, and the animals are weighed about every two weeks.

The Group Condition (GC) is like EC except that no inanimate stimulus objects are placed in the cages. As in EC, the animals are moved from one cage to another each day.

The Complex Maze (CM) condition employs a plastic box inserted to provide two additional floor levels in an EC cage. The maze box was made of clear Plexiglas and measured 10 cm high x 74 cm x 74 cm; it was placed within an EC cage on flanges that supported it 15 cm above the cage floor. Holes 7 cm in diameter were placed at the four corners of the top and bottom of the plastic box so that the rats could crawl into and out of it; any of these holes could be closed with a plastic door when desired. Two of the plastic boxes contained 36 Plexiglas pillars with slots in the

four sides so that Plexiglas panels 10 cm wide could be inserted to form maze patterns. Four other boxes had some barriers permanently in place and other barriers whose positions could be changed to alter the maze patterns; these four boxes provided a different maze pattern daily over a 30-day experimental period. Food pellets were made available, as in EC and GC, on the floor of the cage, but the water bottle was placed above the plastic box so that to get from food to water the rat had to climb into the plastic box at an open corner in the bottom, traverse the box to an open corner at the top, climb out of the box and stand on its top to reach the spout of the water bottle. Rats that eat dry food pellets like to drink frequently, so they had to run up and down a number of times during each bout of feeding; the rats were not able to carry food pellets up into the maze and above it to the water station. The following pretraining schedule was established. On day 1, the rats were placed into the cage without the plastic box present, and both food and water were available on the floor. On day 2, the top of the plastic maze (that is, a plastic sheet with holes at the corners) was placed on the brackets, and the water bottle was placed above it, so that in order to reach the water the rats had to climb through any of the four corner holes and stand on the sheet of plastic. On day 3, the maze box was put into place with all top and bottom holes open; the maze contained a simple pattern of barriers. On day 4, only one bottom and one top hole were left open, so that the rats had to traverse the box.

For the next 29 days, the pattern of barriers was changed daily. Six CM cages were set up adjacent to each other with six different maze patterns; the animals were moved from one cage to another each day, and at the end of each sixth day, all maze patterns were changed. Figure 1 gives examples of some of the maze patterns.

The Simple Maze (SM) condition was like CM with two exceptions: The animals remained in the same cage throughout the experiment, and the same simple pattern of barriers (see the left-hand pattern in Fig. 1) was maintained throughout.

We also used a "superenriched" condition in some experiments, since Kuenzle and Knüsel[9] had reported such a condition to show larger brain effects than EC, and since Davenport[4] had reported the superenriched condition to be more effective than EC in promoting behavioral recovery in neonatally hypothyroid rats. Our Superenriched Condition (SEC) was similar to that of Davenport, which in turn was modified from that of Kuenzle and Knüsel. The setup for SEC was as follows: Three regular EC cages were placed 12 cm apart, and each of the side cages was linked to the center cage by two tunnels. The tunnels had a cross-section of 8 x 8 cm, the sides and top being made of sheet metal and the bottom of hardware cloth. A sheet metal door was hung in the center of each tunnel; it could be propped open or allowed to hang, and it could be prevented from swinging in one direction or the other by a bar placed close to the bottom of the tunnel. The tunnels were located

with their bottom 20 cm above the bottom of the cages and 5 cm in from either the front or back. Stimulus objects were placed in each of the three cages, the toys being changed daily from the usual set of objects. On a number of days, food was placed only in one of the cages and water only in another cage in order to ensure that the animals would explore the whole environment, but observation indicated that the animals explored the area actively whether food and water were separated or not. Forty-eight rats were placed in SEC.

As a control condition for SEC, to test whether the large social group alone would produce enhanced cerebral differences from rats in IC, a condition similar to SEC was set up but without the presence of any inanimate stimulus objects, with no doors in the tunnels, and with food and water always available in each of the three linked cages; this was called the Multiple Cage Condition (MCC). Forty-eight rats were placed in MCC as in SEC.

As a departure from the artificial laboratory conditions, a seminatural environment (SNE) was also tested for its effects on brain measures in experiments that also included the standard EC and IC treatments. The seminatural environment was established in an outdoor "population pit" at the Field Station for Research in Animal Behavior above the Berkeley campus. The population pit is a 9 x 9 m concrete rectangle, filled with earth to a depth of about 30 cm, and with a wire mesh roof. Some stones, branches, and pieces of wood lay on the surface of the dirt, and weeds grew

in it. Four stations for food and water were placed in the pit, and food and water was available at at least one of the stations each day. Twelve animals were placed in SNE.

Most experiments included rats housed singly in the impoverished condition (IC). The cages for IC measure 32 x 20 x 20 cm. The IC rats, like those of the other conditions, are provided with food and water ad libitum, and they are weighed when their littermates are weighed.

Dissection and Weighing of Brain Tissue

At the end of the behavioral phase of an experiment, the animals were put in a multiple-unit cart bearing code numbers that did not reveal the experimental condition of any rat. The animal was decapitated, and the brain was dissected following our standard procedures[22]. Using a calibrated plastic T-square, we removed standard samples of occipital and somesthetic cortex. The other brain sections were the following: remaining dorsal cortex; ventral cortex, including the hippocampus and corpus callosum; cerebellum and medulla; remaining subcortical brain, including the olfactory bulbs. Measures from all of the cortical sections were combined to give rest of brain.

As soon as each sample was removed, it was weighed to the nearest tenth of a milligram on an automatic balance. The samples were then frozen on dry ice and stored at -30°C for subsequent chemical analysis.

Analyses of RNA and DNA

Analyses of RNA and DNA were made according to procedures developed recently in our laboratories and described in detail by Morimoto et al.[11] All operations were carried out at 0-5°C, with cold solutions. Frozen sections of brain were homogenized with a Potter Elvehjem homogenizer in cold ethylenediaminetetraacetic buffer to a concentration of 25 mg/ml. In a 16 x 75 mm culture tube, 4 ml of homogenate were added to 2 ml of 3% cetyltrimethylammonium bromide, and the precipitate was allowed to form. After 1 hr, the precipitate was collected by centrifugation at 7,000 x g for 15 min. The supernatant was discarded, and the pellet was washed twice with 1 ml of H_2O, then once with .1N KOAc in absolute ethyl alcohol. The pellet was centrifuged and dispersed between each washing.

For determination of RNA, the tissue pellet was dispersed with 500 µl of 1.3 N perchloric acid (PCA) and allowed to stand for 15 min at 0°C. After centrifugation at 7,000 x g for 15 min, the supernatant was recovered, and the acid-insoluble fraction was washed twice with 500 µl of .2 N PCA. The three supernatants were pooled, and the volume was adjusted to 5 ml (.1 N PCA). The RNA was assayed by absorbance at 260 nm. The RNA content was calculated on the assumption that an absorbance of 1.00 at 260 nm is equivalent to 32 µg RNA/ml.

The acid-insoluble fraction was then drained and blotted dry. One milliliter of 1 N PCA was added and the pellet was thoroughly dispersed. The residue, which is almost entirely DNA, was heated for 20 min at 70°C, cooled, and spun at 7,000 x g for 15 min. The DNA was determined by absorbance at 266 nm and calf thymus was used as a standard; an absorbance of 1.00 at 266 nm is equivalent to 45 µg DNA/ml. Results for both RNA and DNA are expressed as milligrams/gram of wet tissue weight. Analyses for the larger tissue sections are routinely made in duplicate. In these experiments (N = 113) the mean difference between duplicate analyses was found to be 2.5% for RNA and 4.0% for DNA.

Analyses of AChE and ChE

The quantitative method of Ellman, Courtney, Andres, and Featherstone[5] was adapted for the differential assay of acetylcholinesterase (AChE) and cholinesterase (ChE). Our procedure has been described in some detail in Rosenzweig and Bennett[15]; a complete description can be obtained from the authors upon request.

Analyses for both AChE and ChE are routinely made in duplicate; two AChE values usually agree within 2%, and two ChE values within 3%.

RESULTS

Cerebral Measures

Effects of adding environmental enrichment to social stimulation

Three experiments included the following five conditions--EC, CM, SM, GC, and IC--and also included analysis of RNA and DNA for

several sections of the brain. One of these experiments ran from 35 to 66 days of age (N=11 per condition), one ran from 45 to 74 days (N=11 per condition), and one ran from 70 to 120 days of age (N=12 per condition); since results of the three experiments were closely similar, they have been combined for overall analyses. Other experiments were run that included only three or four of these five conditions, so comparisons have been made among the conditions using larger Ns, but these did not change significantly any of the results; we will therefore restrict the following presentation to the three experiments that included all of the five above named conditions.

Differences in brain weights between rats in EC and IC in these experiments conform to the usual pattern, as indicated in column 3 of Table II (see for comparison Table I of Bennett et al.[2]). That is, significant differences are found in each of the cortical areas measured, with the largest difference occurring in the occipital area, but no difference is found in the rest of the brain. The two maze-experience conditions--CM and SM--also brought about significant differences in cortical weights from animals housed in IC; the distribution of these differences among regions of the brain followed closely the pattern of EC-IC differences, although the effects brought about by maze experience tended to be somewhat smaller than those of EC. In contrast to the effects of living in a varied environment (EC) or of maze experience (CM or SM), simply

living in the same-sized group of 11 or 12 and in the same-size cage (GC) was less effective in altering brain values from those of the IC littermates. GC did show significant differences from IC in weights of occipital and ventral cortex, but not in weights of somesthetic or remaining dorsal cortex; the differences from IC in weights of total cortex and of the cortical/subcortical ratio were highly significant. The effects induced by EC were significantly larger than those induced by GC in three of the four cortical regions measured, in total cortex, in total brain and in the cortical/subcortical ratio (see column 7 of Table II). Experience with varied maze patterns (CM) was also significantly more effective than GC in altering a number of the brain weight measures; experience in a simple unvarying maze pattern (SM) was only marginally more effective than GC in influencing brain weight values.

RNA and DNA were analyzed only in the occipital cortex and in a pooled sample consisting of somesthetic plus remaining dorsal cortex. No analyses were made of noncortical tissue, because in previous work we have not found any significant effects of differential experience on RNA or DNA in noncortical brain. Table III shows that all of the socially grouped conditions brought about highly significant differences from the brain values of littermates kept in the individual IC treatment. The effects of maze experience were almost as large as those of the varied enriched condition (EC), but the effects of grouped living without any special environmental stimulation were only about half as large as those of EC. The fact

that enrichment of the inanimate environment produced significantly larger differences from the IC baseline than did social grouping is shown clearly in the three columns to the right in Table III. Thus, in the case of RNA/DNA as well as in that of weights of cerebral tissues, while living in a social group did produce significant difference from living in isolation, adding the enrichment of inanimate stimulation--whether in the form of varied stimulus objects or in the form of maze patterns to be learned--significantly increased the cerebral effects.

Effects of experience in a seminatural environment

Several different experiments permit comparison between brain values of rats assigned to the usual enriched environment in the laboratory (EC) and an equal number of littermates assigned to the outdoor seminatural environment (SNE). One set of four experiments included littermates assigned to the following three conditions that were all set up at the Field Station--SNE, EC, and IC; all brain sections were analyzed for acetylcholinesterase (AChE) and cholinesterase (ChE) activities. Results from these experiments are presented in Table V, but before examining them, let us look at results of later experiments that remove a possible difficulty in interpreting the Field Station experiments. In the early experiments conducted at the Field Station we noticed that the differences between cerebral values of EC and IC littermates were somewhat smaller than had been obtained in our laboratories in

Tolman Hall, so in subsequent experiments we included groups given EC experience in the standard conditions in Tolman Hall (EC_T) as well as groups given EC experience in the shed at the Field Station (EC_{FS}). Three experiments were run that included the following littermate groups--SNE, EC_{FS}, EC_T, and IC--and in which RNA and DNA were analyzed; in one of these experiments the nucleic acids were analyzed only in occipital cortex, but in the other two they were analyzed both in occipital cortex and in a pooled sample of somesthetic and remaining dorsal cortex.

In terms of weights of brain tissue, the seminatural environment caused development of the brain beyond that found in EC. This is demonstrated by the significant differences between brain values of SNE rats and their littermates in either EC_{FS} or EC_T; see Tables IV and V. Table IV shows that cortical weights for both EC_{FS} and EC_T rats were significantly greater than values for IC littermates and significantly smaller than values for SNE littermates. While the EC_T effects did tend to be somewhat larger than EC_{FS} effects, the values of the two EC groups did not differ significantly from each other, so we can accept as representative the EC_{FS} values reported in Table V. Examination in Table V of the magnitudes of effects in the different cortical regions shows that the pattern of effects induced by SNE was similar to that induced by EC (the largest differences occurring in the occipital area and the smallest in the somesthetic area of the cortex).

In the case of the RNA/DNA ratio, Table IV shows that for both occipital and total cortex the values for SNE rats were significantly greater than those for EC littermates, and the EC values were significantly greater than those of IC littermates. The RNA/DNA data in the table come from the two experiments in which all of the dorsal cortex was analyzed. Data for the occipital cortex in a third experiment somewhat enhanced the differences between the effects of SNE and EC. The greater RNA/DNA cortical values of the enriched experience (SNE or EC) rats versus their IC littermates reflect chiefly lower DNA/weight but also somewhat greater RNA/weight in the enriched experience rats. (DNA/wt decreases with SNE or EC because the cortex grows in bulk without any important change in the number of cells; that is, total DNA in the cortex remains essentially constant, whereas total RNA increases significantly as a result of enriched experience.)

Table V presents results of experiments in which AChE activity was analyzed. The tissue weight effects were closely similar to those of the experiments reported in Table IV and in previous publications. In AChE activity, the differences between EC and IC littermates also followed the pattern of previous experiments, with EC rats showing significantly lower values of AChE/wt in the cortex, especially at the occipital cortex (-3.4%, $p < .001$). The new SNE condition yielded differences from IC values that were similar in distribution to the EC-IC differences but were larger in magnitude;

in occipital cortex the SNE-IC difference amounted to -6.0%, $p < .001$. This was significantly larger than the comparable EC-IC effect ($p < .001$). In the case of cholinesterase, there were few significant effects of environmental treatments, in conformity with earlier findings that it usually requires experiments of greater than 30-day duration to produce significant differences in ChE.

Thus for measures of cortical weights, RNA/DNA, and AChE, giving a group of 12 rats experience in the outdoor seminatural environment produced significantly larger effects than giving 12 littermates experience in EC.

Effects of superenriched environment

Our experiments in which 48 rats were placed in three interconnected cages with varied stimulus objects (SEC), or a similar setup but with no stimulus objects in the cages (MCC), allowed us to test whether the "superenriched" condition would produce significantly larger brain effects than the EC treatment and whether increasing the size of the social group from 12 to 48 would itself be effective in altering brain values. Two experiments were done that included each of the following conditions--SEC, MCC, EC, GC, and IC. In each case, 24 of the animals in SEC and 24 of the animals in MCC were littermates of animals in the other conditions; two EC cages and two GC cages were run in each of these experiments. The other 24 animals in SEC and MCC were fillers. In the second of these experiments, RNA/DNA was analyzed for occipital cortex.

The results of these experiments showed that the superenriched condition did not cause any significant differences in either weights of brain measures or in RNA/DNA of occipital cortex from the values of EC littermates; both SEC and EC were equally effective in producing significant differences from the brain values of IC littermates. The results of the experiments involving the seminatural environment (SNE) showed that the EC values were not at a ceiling, since the SNE brain values were significantly greater; we conclude that the "superenriched" environment, at least in our version of it, was no more effective than EC in altering cerebral values. It should be noted, however, that the condition that included 48 rats in three cages without varied stimulus objects (MCC) was just as effective as the condition with stimulus objects (SEC) in heightening brain values; that is, there were no significant differences between brain values of the littermates in SEC and in MCC. Thus, while in groups of 12 there were clear differences in brain values between animals with exposure to an enriched inanimate environment (EC or CM) and without such exposure (GC), in the case of the larger group of 48 animals living in a larger cage area, the addition of varied stimulus objects did not produce a visible cerebral effect. The apparent difference of effectiveness of inanimate stimulus objects in the smaller and larger social conditions will be considered in the Discussion.

Behavioral Effects

The Introduction to this paper referred briefly to some results comparing effects on several behavior tests of giving animals

enriched experience or housing them in groups of three. The experiment to be reported here provides a more direct test than do the former reports of effects of inanimate stimulation, because it includes both EC and GC groups that are alike in the number of animals housed together and alike in the cage space but that differ only in the availability of varied stimulus objects in EC and their absence in GC. The experiment also investigates the effects of these differential environments both on intact animals and on animals that have had part of the cortex removed in a surgical procedure. We have previously shown that when animals have suffered a lesion in the occipital cortex, subsequent exposure to the enriched condition improves problem-solving performance on the Hebb-Williams maze; this is true whether the lesion is made neonatally[25], shortly after weaning[26], or in animals over 100 days of age[24]. In the present experiment the lesions were inflicted at about 30 days of age and then the animals spent the subsequent 90 days in one of three conditions--the standard colony (SC) treatment with 3 rats in a colony cage, or GC, or EC. Following the end of the 90-day period of differential experience, the animals were reassigned to individual cages and deprived of food pellets. The animal technicians and student volunteers who tested the animals did not know from what environmental condition any animal came or whether or not it had a cortical lesion.

Following a 10-day schedule of pretraining to eat in the goal box and then to leave the start box promptly and find a way to the

goal box of the apparatus, the animals were tested on 10 of the 12 standard problems of the Hebb-Williams maze[12]. Problems 6 and 12 were omitted from the standard series. Eight trials were given on each day of testing. Performance was scored in terms of initial errors and repetitive errors; for initial errors, no more than a single entry in any cul-de-sac was scored per trial, whereas repetitive errors were entries after the first into a given cul-de-sac on a single trial.

Figure 2 shows the main results in terms of mean errors per rat for all 10 problems combined, shown separately for each of the six experimental groups. An analysis of variance showed highly significant effects for both initial and total error scores. Rats with brain lesions made significantly more total errors than intact rats ($F[1,60] = 26.76$, $p < .001$). There was also a clear effect of postlesion environmental treatment on total error scores ($F[2,60] = 9.65$, $p < .001$); this effect was due almost entirely to the difference in scores between the EC rats and the other rats. Among the sham operates, the Least Significant Difference test showed the EC rats to perform significantly better than the SC rats ($p < .05$), whereas the difference between the GC and SC means was not significant. Among the rats with brain lesions, rats exposed to EC performed significantly better than either the GC or SC rats, whereas the difference between GC and SC were not significant. Note in Figure 2 that the scores of EC rats with cortical lesions were almost as good as those of intact animals that had GC or SC experience postoperatively.

In other experiments we have shown that for performance on the Hebb-Williams maze, experience in SC with 3 animals per cage is no more helpful than experience in IC with a single animal per cage. We now see that experience with 12 animals in a cage in GC is also not effective in lowering errors on this test. Thus, for the widely used Hebb-Williams test, experience in social groups does not appear to benefit performance significantly, but adding varied inanimate stimulus objects to the environment brings about a significant improvement in subsequent maze performance.

DISCUSSION

The results of the experiments reported here make it abundantly clear that, while housing animals in a group of 12 in a relatively large laboratory cage leads to cerebral changes in comparison with littermates housed individually in a small colony cage, significantly larger brain effects can be produced by presenting the 12 animals with a more complex environment. Effects somewhat greater than those of the group condition (GC) were obtained simply by requiring animals to traverse a simple maze with an unvarying pattern (SM); still larger effects were obtained in groups that had to traverse a complex maze with a pattern that was changed daily (CM). Somewhat larger effects than those brought about by the maze experience were induced by experience with a few stimulus objects that were changed daily (EC). And still larger cerebral effects were produced by experience in an outdoor seminatural environment. Thus, with group size held constant at 12, the nature of the inanimate environment determines aspects of brain anatomy and brain biochemistry. Simply

housing animals in a group of 12 does bring about significant cerebral differences from individual housing, but these differences are about only half the size of those produced by the enriched condition (EC). In further work[2] we have done away with the social factor entirely by housing animals individually and by giving some animals training in the complex maze or simple maze conditions. Rats given such training individually showed significant changes in weight and in RNA/DNA of the cerebral cortex.

It is worth considering why the presence or absence of stimulus objects did not appear to cause cerebral differences for animals housed in groups of 48 (SEC vs. MCC), whereas the presence or absence of objects did make a difference for rats housed in groups of 12 (EC vs. GC). It may be that with increasing size of social grouping and increasing amounts of social interaction, social stimulation alone is able to bring about a maximal effect. On the other hand, it should be recognized that the effects in these experiments were not at ceiling, because the brain values for the SEC and MCC groups were closely similar to those of EC littermates, while in other experiments we have seen that the EC values lie significantly below those of animals that have been exposed to the seminatural environment (SNE). It may be that the three interlinked cages and tunnels constituted an enriched environment for rats in MCC. In order to get from one of the three cages to another, the animals had to climb 20 cm of the wall, run through the tunnel, and then climb down the other wall. While we have shown that motor

activity as such cannot bring about the sorts of brain changes that we measure, the route from one end cage to the other may have been functionally as complex as the simple maze (SM) pattern. Observations showed that the rats in MCC did go from one cage to another and explore the entire area thoroughly, even though there was no obvious incentive to do so. It now seems that a better way to test the effects of mere social grouping would have been to place the 48 animals in a single cage as large as the three cages together but without the presence of internal walls or tunnels. Thus it does not seem to be necessary to conclude from the present results that as the size of the social group increases, the presence or absence of inanimate stimulus objects becomes less important for full development of the brain, although such an interpretation is certainly possible.

Behavioral results

The results reported here with the Hebb-Williams maze, in which the performance of EC rats was superior to that of both GC and SC rats, resembles the results with the Lashley III maze in which EC rats were found to perform better than either SC or IC rats whose performance was rather similar to each other.[14] In this respect the results with the Hebb-Williams maze and Lashley maze differ from results with Visual Reversal Discrimination[14] and with the tests of Morgan[10] of complex motor skills. Thus, as Yarrow et al.[27] have noted for behavior of human infants, we wish to emphasize for the

performance of laboratory animals that aspects of the environment that may be enriching for one type of problem-solving behavior may not contribute to another aspect of behavior. This point can be extended further by noting that environmental factors that lead to development of brain measures may not lead to development of behavioral measures; we have found that the SNE condition which led to superior brain values did not improve performance on the Lashley III maze.

Factors determining cerebral effects of differential experience

While our results are similar to those of Welch et al.[23] in some ways, there are some major differences. For example, Welch et al. state, "Unlike others, we found no significant differences in the weight ratios of cortex/subcortex[20] or forebrain/hindbrain weight.[3]" Also, their Table II shows animals having lived for a year in a group to have a significantly lower total DNA than isolates, the difference amounting to 6.1%. On the contrary, we find total DNA to be equal or slightly greater in the group animals as compared with isolates. Welch et al. suggest (p. 82) that "... stress or high levels of environmental stimulation may accelerate the natural aging-associated loss of neurons from the brain."

Having seen that social living can induce some changes in the brain, as compared with isolation living, and that even larger effects are produced when varied inanimate stimulation is added to

social stimulation, we should ask what light this may throw on determination of the factors responsible for production of the cerebral effects. A number of different factors have been suggested, and several of these have been investigated. For example, in our first publications on the EC-IC differences[8,21]; we took up the possibilities that differential amounts of locomotor behavior or differential handling might be responsible, and we showed in control experiments that manipulation of these factors did not affect the results; later tests have also served to eliminate these possible factors.[6,18] Welch et al.[23] have suggested that year-long stress in their social condition may accelerate the natural loss of neurons from the brain that accompanies aging. In our experiments of 30-, 60- or 80-day duration, we have not observed signs of stress in either EC or IC animals; furthermore we have found that even adding overt stress to EC or IC did not produce changes in brain weight measures or brain AChE, although it did significantly affect the weight of the adrenal glands.[13] In further experiments, we found that typical EC-IC differences in cerebral weights develop even in hypophysectomized animals, thus ruling out the necessary participation of the pituitary-adrenal axis in producing the cerebral effects of differential experience.[19] We had originally adopted the EC treatment as a way of providing animals many opportunities for learning[21] and a good deal of evidence supports the position that much if not all of the EC-IC brain differences reflect differential

amounts of learning.[1,7,17,20] This position is now supported further in a paper demonstrating that giving maze training to individual rats (eliminating the possible alternative interpretations of social influence or of differential locomotion) produces significant brain effects which are similar in their pattern of regional distribution to those produced in EC.[2] Furthermore the fact that living in a social group also produces brain effects and that they are similar in their pattern of distribution to the effect of inanimate stimulation does not necessarily run counter to the explanation of brain effects as being produced by learning and reflecting, in part, memory storage. Certainly animals that are caged together spend a good deal of time interacting with each other; they learn to recognize individuals with whom they have been caged from other individuals, and they undoubtedly learn much about each other's anatomical and behavioral particularities.

If the cerebral effects of differential experience reflect, in part at least, the formation of long-term memory traces, then it becomes important to consider what are some of the main biosynthetic steps that are involved in this process. This question is taken up elsewhere in a paper on effect of individual maze training on brain anatomy and cortical RNA/DNA.[2]

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Table I

Names of Conditions	N per Condition	Cage Size	Presence of Stimulus Objects or Maze in Cage	Animals Rotated Among Cages Daily
Enriched Condition (EC)	12	Large (75 x 75 x 45 cm)	Stimulus objects changed daily	Yes
Group Condition (GC)	12	Large	No stimulus objects	Yes
Complex Maze (CM)	12	Large	Maze, pattern changed daily	Yes
Simple Maze (SM)	12	Large	Maze, pattern fixed throughout	No
Superenriched Condition (SEC)	48	3 interlinked large cages	Stimulus objects changed daily	No
Multiple Cage Condition (MCC)	48	3 interlinked large cages	No stimulus objects	No
Seminatural Environment (SNE)	12	Outdoor, 9 x 9 m pit	No stimulus objects	No
Impoverished Condition (IC)	1	Small (32 x 20 x 20 cm)	No stimulus objects	No

Table II

Percentage Differences in Brain Weights Among Littermate Rats in Varied Environments

(N = 34 per condition)

	IC	Differences from IC Rats				Differences from GC Rats		
Cortex	$\bar{X}$(mg) ± SD	EC	CM	SM	GC	EC	CM	SM
Occipital	70.0 ± 3.5	6.1****	7.5****	5.3****	2.8**	3.2**	4.6****	2.4*
Somesthetic	58.0 ± 2.4	3.3***	2.6**	2.2*	1.2	2.0*	1.3	0.9
Rem. Dorsal	284 ± 15	4.9****	4.5****	3.3***	1.3	3.6***	3.2***	2.0*
Ventral	253 ± 18	6.4****	6.0****	5.1****	4.6****	1.7	1.3	0.5
Total	666 ± 27	5.5****	5.2****	4.1****	2.7****	2.7****	2.4****	1.4*
Rest of Brain	922 ± 42	0.9	1.9**	0.8	-0.4	1.3	2.3**	1.2
Total Brain	1588 ± 66	2.8****	3.3****	2.2***	0.9	1.9**	2.4***	1.3*
Cortex/Rest	.722 ± .020	4.5****	3.3****	3.3****	3.1****	1.4**	0.2	0.2

*p < .10, **p < .05, ***p < .01, ****p < .001

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Table III

Mean Values for IC Rats and Percentage Differences Among Rats in Varied Environments for RNA, DNA, and RNA/DNA of Cerebral Cortex

(N = 34 per condition)

	IC		Differences from IC Rats				Differences from GC Rats		
Cortical Region:	$\bar{X}$	± SD	EC	CM	SM	GC	EC	CM	SM
A. RNA (μg/100mg)									
Occipital	167.3	4.8	1.6**	1.1*	1.3**	0.3	1.2*	0.7	1.0
Somesthetic + Rem. Dorsal	164.1	3.5	0.2	0.6	0.7*	0.4	-0.2	0.2	0.3
Total Dorsal	164.7	3.2	0.4	0.6*	0.8**	0.4	0.0	0.2	0.4
B. DNA (μg/100mg)									
Occipital	104.5	4.9	-7.5****	-7.1****	-7.0****	-5.2****	-2.3**	-1.9*	-1.8*
Somesthetic + Rem. Dorsal	98.7	4.5	-4.1****	-3.2****	-2.2***	-1.4*	-2.7****	-1.8**	-0.8
Total Dorsal	99.7	3.9	-4.7****	-3.9****	-3.0****	-2.1***	-2.7****	-1.8***	-1.0
C. RNA/DNA									
Occipital	1.616	.055	9.8****	8.5****	9.2****	5.8****	3.8****	2.6***	3.2****
Somesthetic + Rem. Dorsal	1.666	.068	4.4****	4.0****	3.0****	1.8***	2.5****	2.1***	1.2*
Total Dorsal	1.653	.057	5.3****	4.7****	4.0****	2.5****	2.7****	2.2****	1.5**

*p < .10, **p < .05, ***p < .01, ****p < .001

Table IV

Percentage Differences of Brain Weights and RNA/DNA Between Littermates in Enriched (SNE or EC) or Impoverished Conditions (IC)

	Differences from IC			Differences from SNE	
	SNE	EC_T	EC_{FS}	EC_T	EC_{FS}
A. Brain Weights (N = 35 per condition)					
Occipital Cortex	11.4****	8.5****	5.9****	-2.7*	-5.2****
Total Cortex	7.4****	4.2****	2.0**	-3.1***	-5.3****
Rest of Brain	-0.1	-1.0	-2.6***	-1.0	-2.6***
Cortex/Rest	7.5****	5.2****	4.7****	-2.1****	-2.6****
B. RNA/DNA (N = 23 per condition)					
Occipital Cortex	12.4****	10.0****	9.2****	-2.2**	-3.0***
Total Cortex	5.2****	3.7****	2.6****	-1.4**	-2.4***

*$p < .10$, **$p < .05$, ***$p < .01$, ****$p < .001$

Table V

Mean Values for IC Rats and Percentage Differences in Weight and Acetylcholinesterase of Brain Samples Among Littermates in EC, SNE, or IC Conditions

	Tissue Weights[a]					AChE/weight[a,b]				
			Percentage differences					Percentage differences		
	IC		EC vs.	SNE vs.	SNE vs.	IC		EC vs.	SNE vs.	SNE vs.
	$\bar{X}$(mg)	± SD	IC	IC	EC	$\bar{X}$	± SD	IC	IC	EC
Cortex										
Occipital	65	3.9	6.0****	11.2****	5.0****	61	2.8	-3.4****	-5.9****	-2.6***
Somesthetic	55	2.7	1.8**	2.1**	0.3	75	2.7	-0.8	-2.0***	-1.2*
Rem. dorsal	282	13.7	3.1****	6.1****	3.0****	78	3.4	-1.5**	-3.2****	-1.7*
Ventral	258	17.0	1.3	4.8****	3.4***	120	5.9	-1.6*	-3.3****	-1.7*
Total	660	25.8	2.6****	5.8****	3.1****	93	3.8	-1.9***	-3.6****	-1.8**
Rest of Brain	881	41.1	-0.8	-0.7	0.1	198	7.8	1.2**	1.2*	0.2
Total brain	1541	64.5	0.6	2.1****	1.4**	153	5.3	-0.2	-1.1**	-1.0*
Cortex/Rest	.750	.019	3.3****	6.5****	3.1****	.467	.014	-3.0****	-4.7****	-1.8***
Body (g)	230	18.8	-7.9****	-10.1****	-2.4**	--	--	--	--	--

[a] N = 47 per condition

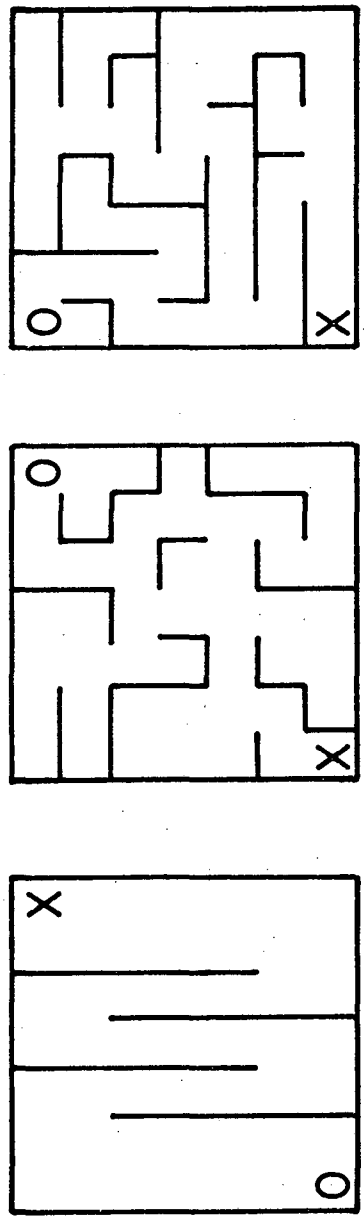
[b] AChE activity is expressed in units of nanomoles acetylthiocholine hydrolyzed/min/mg tissue.

*p < .10, **p < .05, ***p < .01, ****p < .001

CAPTIONS

Figure 1. Examples of the patterns of barriers used in the plastic maze boxes. O indicates a door open in the bottom of the maze box; X indicates a door open in the top. The pattern at the left was that maintained throughout the experiment in the Simple Maze condition.

Figure 2. Mean errors per rat on trials 2-8 for 10 problems of the Hebb-Williams maze. After either a sham operation or bilateral removal of occipital cortical tissue at about 30 days of age, rats spent the next 90 days in one of three environments: Enriched Condition (EC), Group Condition (GC), or Standard Colony Condition (SC).



XBL 777-4502

Fig. 1

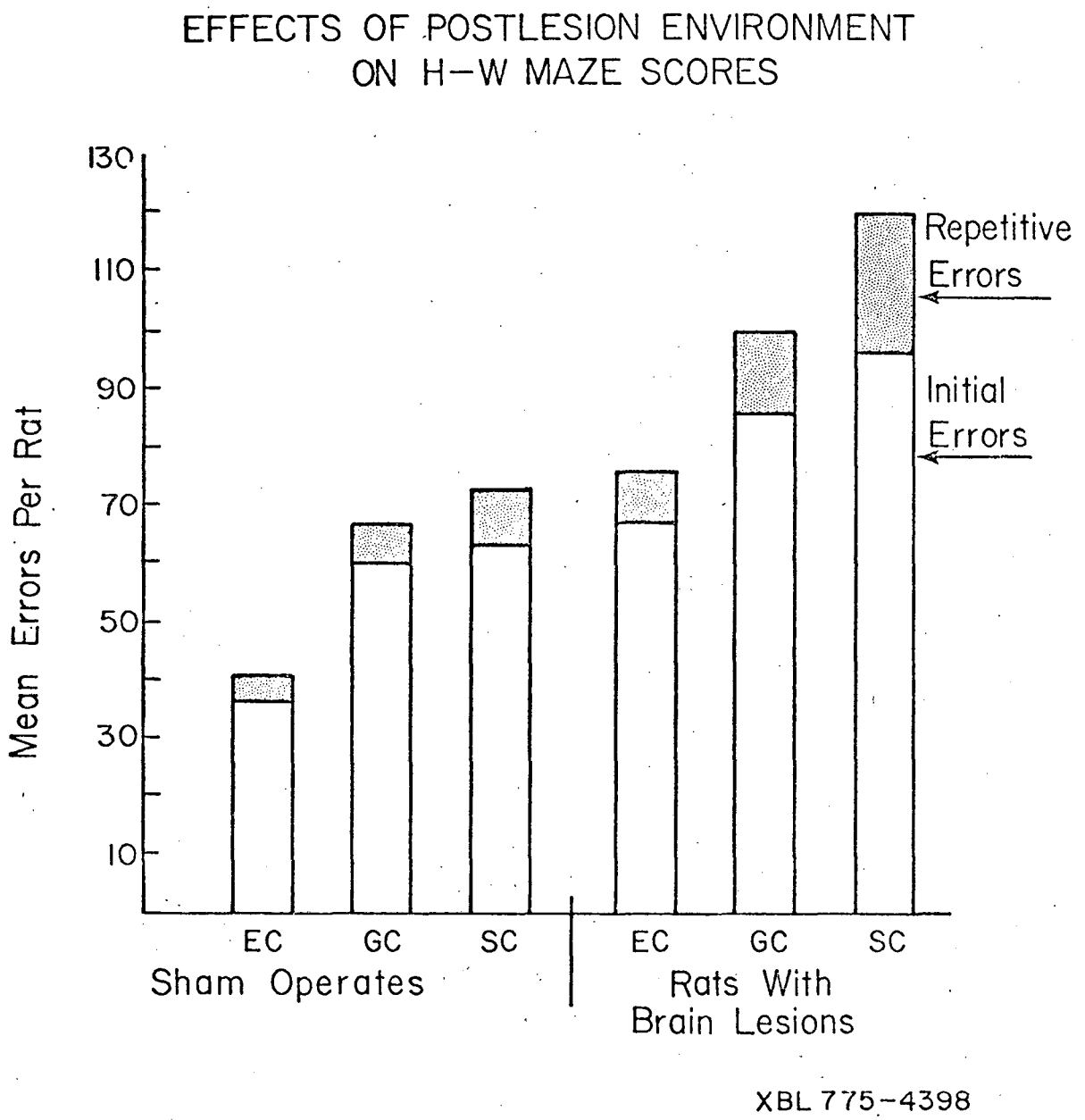

EFFECTS OF POSTLESION ENVIRONMENT
ON H-W MAZE SCORES
Mean Errors Per Rat
130
110
90
70
50
30
10
Repetitive Errors
Initial Errors
EC
GC
SC
Sham Operates
EC
GC
SC
Rats With Brain Lesions
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Fig. 2

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