Title

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Author

Bennett, Edward L.

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Bruno E. Will, Mark R. Rosenzweig, Edward L. Bennett, Marie Hebert, and Hiromi Morimoto

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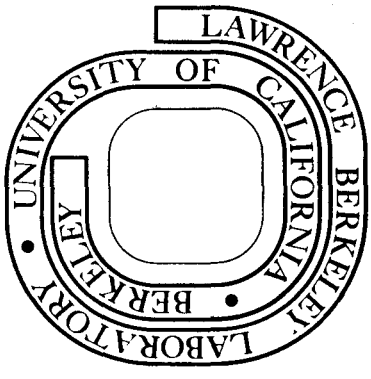

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Relatively Brief Environmental Enrichment Aids Recovery of Learning Capacity and Alters Brain Measures after Postweaning Brain Lesions in Rats

Bruno E. Will

Laboratoire de Psychophysiologie

Université Louis Pasteur, Strasbourg, France

Mark R. Rosenzweig

University of California, Berkeley

Edward L. Bennett, Marie Hebert, and Hiromi Morimoto

Lawrence Berkeley Laboratory, Berkeley

Running head: Environmental Enrichment Aids Recovery of Learning Capacity

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Bruno E. Will
Université Louis Pasteur, Strasbourg, France

Mark R. Rosenzweig
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Edward L. Bennett, Marie Hebert, and Hiromi Morimoto
Lawrence Berkeley Laboratory, Berkeley

ABSTRACT

Enriched postlesion experience aided in overcoming effects of simultaneous bilateral cerebral lesions made at 30 days of age in one experiment with inbred Fischer rats and in a second with the Berkeley S_1 strain. The lesions were directed to the occipital cortex, but in most cases there was also some impairment of the hippocampus. For 60 days after operations, half of the rats lived in small individual cages and half lived in groups in large enriched-environment cages. They were then pretrained and tested on the standard 12 Hebb-Williams problems. Daily injections of methamphetamine (vs. saline) in the first experiment produced no effect on the behavioral scores. The second experiment included groups that received only 2 hr/day of enriched experience, and they benefitted as much as groups that remained in the enriched environment 24 hr/day. The results of both experiments demonstrate significant beneficial effects of environment when bilateral lesions were made at a later age and when the periods of enriched experience were shorter than have previously been tested. A third experiment, run in parallel with the second behavioral experiment, revealed

significant effects of both lesions and environment on both weight and RNA/DNA of brain regions.

Reprint requests should be sent to either Bruno E. Will, Laboratoire de Psychophysiologie, Université Louis Pasteur, Strasbourg, France, or to Mark R. Rosenzweig, Department of Psychology, University of California, Berkeley, California 94720.

The role of environmental or pharmacological stimulation in aiding recovery from effects of brain lesions has been studied relatively little, and their value remains controversial for both animal subjects (Greenough, Fass, & DeVoogd, 1975; Isaacson, 1975) and human patients (Sarno, 1970; Stern, McDowell, Miller & Robinson, 1971; Teuber, 1974). While stimulation in the period between two unilateral lesions has been reported to be beneficial in several experimental studies (e.g., Petrinovich & Carew, 1969; Kircher et al., 1970), effects of stimulation after bilateral lesions have rarely been investigated, even when simultaneous bilateral lesions were included in the same experiments as successive unilateral lesions (e.g., Petrinovich & Bliss, 1966; Petrinovich & Carew, 1969; Kircher et al., 1970). Many investigators seem implicitly to have concluded that stimulation after simultaneous bilateral lesions would be ineffective and a waste of effort. Exceptions to the latter conclusion are the results reported briefly by Smith (1959) and the often cited single experiment of Schwartz (1964). Schwartz made bilateral posterior cortical lesions in rats during their first postnatal day. Lesioned rats and sham-lesioned controls were then raised from day 5 until day 95 in either impoverished or enriched environments. When the rats were subsequently tested in the Hebb-Williams maze, both brain status (lesioned vs. sham) and environment yielded significant effects; there was also a significant interaction in that enriched environment caused a greater absolute reduction of errors among the lesioned than among the control rats. Early enriched experience offset the effects of the lesions so strongly that lesioned rats from the enriched environment made fewer errors than intact rats from the impoverished environment.

We have now obtained results rather similar to the interesting findings of Schwartz with neonatal lesions (Will, Rosenzweig, & Bennett, in pre-

paration), and in the present paper we have extended this research to take up the following questions: Can enriched experience still aid recovery if the bilateral lesions are inflicted at a later age than day 1? Must the enriched experience be maintained for 90 days, or will a shorter period suffice? Must the enriched environment be available 24 hr/day, or can a brief daily period of environment "therapy" be effective? The two experiments reported here are part of a series directed to this problem. In both experiments bilateral occipital cortical lesions were inflicted at about 30 days of age. The subsequent period of environmental enrichment or impoverishment lasted 60 days, about two-thirds as long as in Schwartz's experiment. In Experiment II some groups were placed in the enriched environment for only 2 hr/day during the 60-day period.

Based on findings that stimulant drugs can enhance the cerebral effects of environment enrichment (Rosenzweig & Bennett, 1972; Bennett, Rosenzweig & Wu, 1973) and on use of stimulants to aid recovery from brain lesions (e.g., Ward & Kennard, 1942; Cole, Sullins & Isaac, 1967), we have suggested investigating the efficacy of combining an enriched environment and a stimulant drug to promote recovery (Bennett et al., 1973, p. 327). For this reason the first experiment employed a drug-nondrug treatment in combination with environment and brain lesions.

Experiment I

Methods

Subjects. Sixty-four male rats of the Fischer inbred strain were obtained from Simonsen Laboratories at about 28 days of age; they had been weaned two or three days before delivery. They were assigned at random before operation to 8 treatment groups (drug x operation x environment). Of the 8 assigned to each group, the numbers surviving through behavioral

testing are shown in Figure 3.

Surgery. The operations took place two or three days after receipt of the rats. The animals were anesthetized by injection of a mixture of chloral hydrate plus pentobarbital sodium. The skull was exposed and an opening about 2 mm diameter was made by drilling over the occipital area of each hemisphere. Cortical tissue was removed by gentle suction. Histology later showed that in all rats some subcortical impairment occurred, at least unilaterally. The sham operates were anesthetized and the skin opened as for the experimentals, but the skull was left intact. After the operations, which were done over a period of 3 days, animals were placed in individual colony cages. Four days after the last set of operations, the rats were placed in the experimental environments; they were about 36 days of age at this point.

Environmental treatments. Half of the animals had been preassigned to the standard Berkeley impoverished condition (IC) and half to the enriched condition (EC). In brief, the IC rats lived in individual cages (32 x 20 x 20 cm) in a separate isolation room, whereas the EC rats were housed in groups of about 12 in large cages (70 x 70 x 46 cm) furnished with about 6 stimulus objects. Half the rats in an EC cage were lesioned and half were controls. Each EC group was moved from one EC cage to another daily, to provide a different arrangement of stimulus objects. For a fuller description of the EC and IC environments see Rosenzweig and Bennett (1969).

Drug treatments. Beginning on the second day that they were in the differential environments, the rats received a daily I.P. injection between 9 a.m. and noon. Half the rats received 2 mg/k of methamphetamine in 1 cc

physiological saline per 100 g body weight; as the rats became larger, the amount of saline was reduced to 1 cc/200 g body weight. Half the rats received a similar amount of saline but no drug. Among the EC rats, one cage received methamphetamine and the other cage was given saline injections.

Behavioral testing. After 60 days in the differential environments, the rats were weighed and placed in individual cages with water but without food. Henceforth their only food was mash available in the goal box, and body weight was brought down gradually to 80 percent of the value at the start of pretraining. The experimenters who trained and tested the animals did not know to which group any animal belonged. Following a standardized procedure, rats were pretrained over 12 days; at the end of this time they ran through simple practice problems readily, 8 trials/day. They were then tested on the 12 standard Hebb-Williams problems (Rabinovitch & Rosvold, 1951), one problem per day and 8 trials/problem. Three apparatuses were used, in three different test rooms, and almost equal numbers of rats from each condition were tested in each room. Initial and repetitive errors were scored, and running time was recorded. (An initial error is the first made in a given error zone on a given trial; repetitive errors are further errors made in the same zone on a given trial.)

Sacrifice and histology. Eight days after the conclusion of behavioral testing, the rats were sacrificed by decapitation. The brain was removed, the dorsal surface was photographed, and then the brain was placed in 10 percent formalin. From decapitation to placement in formalin, the elapsed time was about 4 min. Later the brains were sectioned with a freezing

microtome. Two days before sectioning, the brains were rinsed and put in a 30 percent sucrose solution. Sections were 50 micra thick and were made perpendicular to the base of the brain. Every tenth section in the region of the lesion was mounted on a slide and used as a photographic negative to obtain enlarged prints of the lesion. Figure 1 shows the extent of the lesions and also indicates the total error score for each animal (sum of errors, trials 2-8, summed over all 12 problems). Some subcortical tissue (corpus callosum and hippocampus) was found to be damaged or removed in all rats, as Schwartz also reported. Typical examples are shown in Figure 2.

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Figures 1 and 2 around here

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Results

The maze scores revealed significant effects of both the lesions and the environmental conditions, different scores bringing out different aspects of the performance. An overall picture is given by Figure 3 which presents total errors per rat on trials 2-8 for all 12 problems. This total error score shows significantly more errors in lesioned than in sham-operated rats ($P < .001$, based on analysis of variance), significantly more errors in IC than in EC ($P < .001$), and a significant interaction ($P < .005$) with the effect of environment being larger among lesioned rats than among the sham-operates. No effect of drug vs. saline was obtained; in fact, the adjacent drug and saline columns for a given lesion-environment combination show the high reproducibility of the test scores for a given condition. When the drug-nondrug treatment is ignored, the four treatment groups (EC-S, EC-lesion, IC-sham, IC-lesion) all differed significantly from

each other on total errors, trials 2-8; all of these differences were significant beyond the .001 level except for EC-lesion vs. IC-sham where the difference reached only the .05 level.

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Figure 3 around here

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Examination of the first trial scores, summed for all 12 problems, yielded an additional finding. The first trial scores showed a significant lesion effect ($P < .001$), but the environmental effect was too small to attain significance. That is, on trial 1, the EC-lesion rats made almost as many errors as the IC-lesion group, and both lesion groups made significantly more errors than the sham-operated groups. On trials 2-4, the EC-lesion rats performed almost as well as the sham-operate groups, whereas the IC-lesion group continued to lag behind.

Analyses of initial and repetitive errors are shown in Table 1. In the case of initial errors, on trial 1 the EC-S group differs significantly from all the others. On the following trials, all 4 groups differ

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Table 1 about here

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significantly from each other, the largest differences occurring between IC-L and the other groups. In the case of repetitive errors, after trial 1 the only significant differences are those between IC-L and the other groups; after trial 1, EC-L has ceased to differ significantly from the sham-operated groups.

Experiment II

Methods

Since the methods of Experiment II were in most respects similar to those of Experiment I, only the differences will be noted here.

Subjects were 60 male rats of the Berkeley S_1 strain (descended from Tryon maze-bright rats). The size of the lesions was somewhat smaller than in Experiment I, as shown in Figure 4, but depths of lesions were similar

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Figure 4 around here
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in the two experiments. All rats but one in Experiment II showed some impairment of subcortical matter, at least in one hemisphere. Since the drug treatment was totally ineffective in Experiment I, it was not included here. Two new groups were added to test the effect of 2-hr daily EC with lesioned and sham-operated rats. Thus there were 6 groups, as shown in Figure 5; the number tested in each group is stated in the figure.

Results

Here, as in Experiment I, total errors per rat on the last 7 trials of all 12 problems (Figure 5) yielded a significant difference between lesion and sham ($P < .001$) and a significant effect of environment ($P < .05$).

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Figure 5 around here
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Interaction between lesion and environment failed to show statistical significance, even though both EC-lesion groups differed significantly from IC-L (2-hr EC-L vs. IC-L, $P < .001$; 24-hr EC-L vs. IC-L, $P < .01$, by Duncan's multiple range test), whereas neither EC-S group differed significantly from IC-S. This was, in fact, the only experiment not to show an EC-S vs. IC-S difference among the five experiments in this series (for

the other three, see Will, Rosenzweig & Bennett and Will & Rosenzweig). The 2-hr EC treatment proved to be slightly more effective than 24-hr EC in reducing errors, but the difference between 2- and 24-hr EC was not significant. Comparisons between each pair of groups for total errors, trials 2-8, are presented in Table 2.

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Table 2 around here
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As we saw in Experiment I, on the first trial of all problems only a lesion effect was found ($P < .01$); all lesioned groups performed significantly worse than all sham-operated groups. After trial 1, the lesioned EC rats (both 2-hr and 24-hr)improved rapidly, whereas the IC-lesioned rats improved much more slowly.

Analysis of initial and repetitive errors in Experiment II, shown in Table 3, yields findings rather similar to those obtained for Experiment I (Table 1). It should be noted that the inclusion of the 2-hr EC groups here complicates Table 3 in comparison to Table 1. The 2-hr EC groups do

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Table 3 around here
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not differ significantly from the corresponding 24-hr groups. The IC-S group here does not differ from the EC-S groups, although it did in Experiment I and IC-S also differed from EC-S in two experiments with neonatal lesions reported elsewhere (Will et al.).

Experiment IIA

Methods

A companion experiment to II was run simultaneously in order to determine effects of the treatments on brain weights and brain RNA/DNA values. The subjects of Experiment IIA were 60 male S_1 rats assigned to the following groups: EC-S, EC-L, IC-S, IC-L. The lesions and environmental treatments were as close as possible to those of Experiment II. At the end of the 60-day period of differential experience, the subjects of Experiment IIA were not pretrained and tested; they were sacrificed for brain analyses. The brain was dissected in a manner close to that employed in other experiments in which we have performed chemical analyses (Rosenzweig, Krech, Bennett & Diamond, 1962). In brief, the rat was decapitated and the calvarium and then the dura mater were removed. A small calibrated plastic T-square was then placed on the dorsal surface of the brain in order to demarcate standard samples of the occipital and somesthetic cortex (see Figure 6). The somesthetic samples (S) from both hemispheres were circumscribed with a scalpel, peeled from the underlying white matter, placed on a preweighed and numbered piece of waxed paper, weighed on an automatic balance to the nearest 0.1 mg, and then placed on their paper on dry ice. The same procedure was followed for the occipital sample, except that the occipital sample in the present experiment was made larger in both the anterior and posterior directions than in our other experiments; this was done in order to be sure to include the area of the lesion within the occipital section. Figure 6 shows the difference in the occipital section between this experiment (O′) and our previous work (O). After removal of the S and O′ samples, the brain was dissected into the following further sections: remaining dorsal cortex; "ventral cortex,"

including not only cortex but also the corpus callosum and the hippocampus; cerebellum and medulla; and the rest of the cerebral hemispheres. The results of the first four samples were later summed to yield values for Total Cortex; similarly the latter two samples were summed to yield a fraction called wither Subcortex or Rest of Brain. To consider cortical weights without influence of the lesioned occipital area, we also summed the other three cortical regions to give Total Cortex minus Occipital or TC-O'. The dissection required about 6 min per animal. The weighed samples were stored in a deep-freeze until chemical analyses were made.

Analyses of RNA and DNA were made according to procedures developed recently in our laboratories (Morimoto, Ferchmin & Bennett, 1974) and described briefly here: All operations are carried out at 0°C. Frozen sections of brain are homogenized using a Potter Elvehjem homogenizer in cold EDTA buffer to a concentration of 25 mg per ml. In a 16 x 75 mm culture tube, 4 ml of homogenate are added to 2 ml of 3% cetyltrimethylammonium bromide (CTAB), and the precipitate is allowed to form. After 1 hr, the precipitate is collected by centrifugation in a Sorval RC-3 centrifuge at 7,000 x g for 15 min. The supernatant is discarded, and the pellet is washed twice with 1 ml H_2O, then once with 0.1 N KOAc in absolute ethyl alcohol. The pellet is centrifuged and dispersed between each washing.

RNA fraction. The tissue pellet is dispersed with 500 µl of 1.3N 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 is recovered, and the acid-insoluble fraction is washed 2x with 500 µl of 0.2 N PCA. The three supernatants are pooled, and the volume adjusted to 5 ml (0.1 N PCA). RNA is assayed by absorbance at 260 nm. The RNA content is calculated

in the assumption that an absorbance of 1.00 at 260 nm is equivalent to 32 ug RNA/ml.

DNA fraction. The acid-insoluble fraction is drained and blotted dry. One ml of 1 N PCA is added, and the pellet is thoroughly dispersed. The DNA is heated for 20 min at 70°C, cooled, and spun at 7,000 x g for 15 min. The DNA is determined by absorbance at 266 nm and calf thymus is used as a standard; an absorbance of 1.00 at 266 nm is equivalent to 45 ug DNA/ml. Results for both RNA and DNA are expressed mg/gm wet tissue weight.

Results

Environmental effects

Brain weights and RNA/DNA values for the sham operates (Table 4) showed effects of environment similar to those that we have observed in

- - - Table 4 about here - - -

other EC-IC experiments (Bennett, 1975). That is, EC-S exceeded IC-S in weights of all cortical sections, especially occipital cortex (7.4 percent, $p < .01$), while the subcortex or Rest of Brain showed no effects. The ratio of weight of total cortex to weight of the rest of the brain (TC/Rest) usually provides especially reliable and significant EC-IC differences, and this was observed in the present experiment, whether or not the lesion sample was included in total cortex. With regard to the RNA/DNA ratio, the sham operates showed somewhat smaller effects than we usually observe: For example, in the present experiment, the EC-IC difference in occipital cortex was 4.9 percent ($p < .01$), whereas in two recent 60-day EC-IC experiments we obtained differences of 12.1 percent and 9.8 percent ($p < .001$ for each). It should be noted that in occipital cortex the RNA/DNA values were not markedly more variable among the lesioned rats than among the sham operates.

Whereas for brain weights the environmental effects were clearly smaller among the lesioned rats (EC-L vs. IC-L) than among the sham operates (EC-S vs. IC-S), the RNA/DNA effects were somewhat larger among the lesioned than among the sham operates, although there was not significant interaction. Thus, for example, the lesioned rats did not show significant EC-IC differences in any of the cortical weights, although they did show a significant effect in the cortical/subcortical weight ratio. On the other hand, for RNA/DNA the EC-lesioned rats showed significantly greater values than IC-lesioned in occipital cortex, somesthetic cortex, total cortex, and in the cortical/subcortical ratio.

Lesion effects

Turning to effects of lesions in the right half of Table 4, we see obvious effects on the weight of the occipital area from which tissue was removed, and it should be noted that the reduction of tissue was virtually equal for the EC and IC groups (31 and 26 mg respectively). There was also a significant secondary effect of the lesions on the remaining dorsal cortex where weight fell by about 6 percent among both EC and IC rats ($p < .01$). The RNA/DNA ratio was reduced in the lesioned rats in all parts of the dorsal cortex (occipital, somesthetic, and remaining), but these changes were small and for the most part, nonsignificant. In striking contrast, the ventral cortex showed significantly greater RNA/DNA in the lesioned than in the intact rats. This unexpected effect (environmental enrichment usually has no effect on RNA/DNA of ventral cortex) may be an indication of compensation for the lesion. This increase of RNA/DNA as a consequence of lesioning was greater in the EC-L than in the IC-L rats, and this also led to greater effects in total cortex and in the cortical/subcortical ratio among EC-L as compared with EC-S.

DISCUSSION

Lack of effect of methamphetamine

From our finding that methamphetamine augments the effects of an enriched environment on brain measures in rats (Rosenzweig & Bennett, 1972; Bennett, Rosenzweig & Wu, 1973) we had suggested that a combination of excitant drug and enriched condition (EC) might be especially favorable for recovery from brain lesions. Cole et al. (1967) had also reported that d-amphetamine overcame the effects of impoverished experience on retention performance of an avoidance response.

In fact, no effect of giving methamphetamine daily during 60 days was found on the Hebb-Williams scores; this was true both for EC and IC and for both lesioned and sham-operated rats (see Figure 3). Let us describe briefly our attempts to account for the lack of effect, since this will lead to a somewhat different hypothesis to be tested in further research. That fact that no group showed an effect helps to eliminate certain possible explanations. For example, there are reports that excitant drugs may interact with brain lesions: Glick and Zimmerberg (1972) reported hyposensitivity of frontally-lesioned mice to d-amphetamine, but several investigators have reported hypersensitivity of frontally-lesioned rats to d-amphetamine (Glick, 1970; Adler, 1961; Lynch et al., 1969). But since our sham-operated rats also did not show an effect of methamphetamine, there is less reason for concern that our lesioned rats may have shown hyposensitivity or hypersensitivity to the drug. During the course of the 60 days of injection, the drug was observed to increase activity of the EC group. Regular observations of activity were made in both the EC-drug and EC-saline groups every 8 days; activity was recorded every 10 min for the 3 hr following injection. The effect of the drug did not diminish during the 60-day

period of injection; the measures of activity showed as large differences between EC-drug and EC-saline during the last weeks as during the initial weeks.

The most probable reason for lack of drug effect in this experiment is that the drug was administered only once a day although the rats remained in EC 24 hr/day, and the greater activity observed in the EC-drug rats following injection was compensated for by their reduced activity, compared with the EC-saline group, during the night. When this possibility occurred to us, after the completion of the experiment, we tested it by observing male S_1 rats every 15 min through two consecutive 24-hr cycles. These rats had been placed in groups of 12 in EC cages 6 days before the observations, and they had been injected with methamphetamine or saline at 8 a.m. each day for 4 days before the observations. Both days yielded similar data. The combined results, presented in Figure 7, show clearly that although the methamphetamine group was more active during the several hours following injection, around midnight the saline-injected group became the more active. Total activity over the complete 24-hr cycle was scarcely greater for the EC-drug than for the EC-saline group.

- - -Figure 7 about here- - -

In our previous experiments in which rats were placed in EC for only 2 hr/day, methamphetamine did induce larger brain effects of EC-methamphetamine than were found in the EC-saline group. In that case, however, the diminished nighttime activity of the 2-hr EC-drug group occurred in the individual cages (IC) where there was little to be gained from the environment. It is only activity in direct contact with the enriched environment that produces the EC effects, as was found by Ferchmin, Bennett and Rosenzweig (1975). It would now appear worthwhile to conduct an experiment on recovery from brain lesions in which 2-hr daily EC was coupled with injections of either methamphetamine or saline. This would examine whether environmental "therapy" that was available for only a limited daily period could be rendered more effective if the

"patients" were made more active and alert during the therapy periods.

Similarities and differences between our results and those of Schwartz

Since the basic design of our experiments is similar to that of Schwartz, it is worth examining similarities and differences in results of the two studies. Like Schwartz, we found significant effects of both lesions and environments on the Hebb-Williams scores, and also a significant interaction in that the effects of environment were significantly larger among the lesioned than among the sham-operated subjects. In Schwartz's experiment, the effect of environment was actually larger than the effect of brain lesions--his EC-lesioned group made fewer errors than his IC-sham group. In our case, the compensatory effect of enriched experience was less complete; the EC-lesioned groups did not perform as well as the IC-sham groups. The relative performances varied according to the behavioral measure employed. Trial 1 errors showed little effect of environment, whereas on trials 2-8 the EC-lesioned rats approached closer to the scores of the sham-operated groups. When running times of Experiment I were analyzed, the EC-lesion group showed slightly (but not significantly) better performance than the IC-sham group. (Mean running times summed for the last 7 trials on all 12 problems were as follows: EC-sham, 389.6 sec; EC-lesion, 599.5; IC-sham, 606.9, and IC-lesion, 986.2.) In Experiment II, however, the EC-lesion groups showed somewhat greater scores than the IC-sham rats (2-hr EC-sham, 376.8; 24-hr EC-sham, 431.0; IC-S, 439.5; 24-hr EC-lesion, 512.3; 2-hr EC-lesion, 574.1; IC-lesion, 936.2).

A number of possible reasons might be suggested for the lesser effectiveness of environment in our experiments than in that of Schwartz. Here are three such possibilities: (a) It is possible that greater recovery can occur after neonatal lesions (as in his experiment) than after lesions inflicted on already weaned rats.

We did not find particularly impressive recovery after neonatal lesions, but our experimental design involving neonatal lesions differed in certain other respects from Schwartz's experiment, as we discuss elsewhere (Will, Rosenzweig & Bennett, Note 1). (b) The greater length of the EC-IC period employed by Schwartz may also have increased the magnitude of the environmental effects. With regard to cerebral effects of EC-IC, we have found some to reach full size within 30 days (e.g., cortical weight) whereas others require more time(e.g., ChE activity). The cerebral correlates of maze learning and of its recovery remain to be determined, as does the influence of duration of differential experience on the recovery. (c) The particular forms of enriched experience given by Schwartz may have been more effective than those that we used. We are inclined to doubt this hypothesis because, in our laboratory, it has taken major modifications in the EC situation to modify significantly the cerebral effects (Rosenzweig & Bennett, in press); perhaps, however, the behavioral effects of different kinds of EC can be differentiated more readily.

Running counter to all three possibilities in the preceding paragraph is the fact that we did find environmental effects to be stronger than lesion effects in an experiment in which rats sustained cortical lesions at about 120 days of age and then spent 60 days in either our EC or IC environments (Will & Rosenzweig, (Note 2)). That is, a greater environmental effect on recovery was found with adult than with young rats, a shorter recovery period than that of Schwartz was effective, and our EC environment led to substantial improvement of performance.

Alternative explanations for differences in performance among the groups

Motivation. To consider the possibility that motivation for food may have differed among groups and may account for part of the differences in behavioral scores, we measured the amount of mash consumed in the goal box for all rats in Experiment II. Soulairac (1952) reported that cortical lesions in rats cause significant differences in consumption of standard laboratory food, glucose, and water. We found no significant differences in consumption of mash among our groups, so that differences in food motivation are unlikely to have caused the observed differences in maze performance.

Exploratory tendency. Since differences in tendency to explore could have affected error scores in the maze, we tabulated each instance of exploration during testing. Trial 1 was not counted, since exploration is expected then. Relatively few cases of exploration were observed; the mean in Experiment II was only 3.0 instances per rat over the 7 last trials of all 12 problems (thus, 3 occurrences out of 84 opportunities). Probably the tendency to explore in the apparatus had been habituated during the extensive pretraining. The largest mean per animal was found in the IC-S group (4.4) and the smallest was in the 24-hr EC-L group (1.9). An analysis of variance revealed no significance related to brain status, environmental treatment or their interaction. Both the lack of significance and the low amount of exploration indicate that this factor cannot account for the differences in maze performance found among the groups.

Does environmental stimulation aid recovery?

Are the effects of environment and of lesions independent and additive, or does it appear that enriched environment helps to compensate for effects of lesions? The effects of lesions on learning or problem-solving behavior have been shown in some studies; the effects of differential experience on such behaviors have been demonstrated in other studies. When both lesions and post-lesion experience are combined in the same experiment, are the effects of these two treatments simply additive or is there a significant interaction? Schwartz (1965) found a significant interaction ($P < .05$); the differential environments had a greater effect on his lesioned than on his control rats. In the first of the present experiments, we found a highly significant interaction ($P < .005$) between effects of lesion and of environment; here too the difference between environments had a considerably larger effect among the lesioned rats than among the sham-operates. In Experiment II, there was also a highly significant effect of environment among the lesioned rats but only a small and nonsignificant effect among the sham-operates. (Environmental treatment did not show a statistically significant interaction with lesions in this case because two of the three treatments were EC--2-hr and 24-hr EC-- and both showed similar differences between the sham-operated and lesioned conditions, whereas IC showed a much larger increase of errors with lesioning. When the error scores were "purified" by removing errors made during obvious exploration, then interaction was found between the three environmental treatments and lesions at beyond the 0.10 level of confidence.) It thus appears that in these experiments the effects of post-lesions environment and of lesions are not simply additive; environment has a greater effect on the lesioned than on the normal rats. Our confidence in the generality of this conclusion is tempered by the fact that our other experiments in this series have not shown clear evidence of interaction.

Granted that the effects of environment are generally, if not uniformly, stronger among lesioned than among normal rats, we may consider briefly possible mechanisms. One possibility is that stimulation may aid functional recovery of damaged tissue. Evidence for the beneficial effect of direct stimulation of the brain comes from quite a different situation: Horrell, Raubeson and Balagura (1974) found that after lesions of the lateral hypothalamus, one hour per day of weak electrical stimulation of this region shortened the time required for recovery of feeding from 5.8 days for nonstimulated rats to only 2.4 days. A hypothesis to be examined in further research is that enriched experience, as well as being of benefit for cognitive development and for brain development among intact individuals, also aids functional recovery after damage to the brain.

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References

Adler, M. W. Changes in sensitivity to amphetamine in rats with chronic brain lesions. Journal of Pharmacology and Experimental Therapeutics, 1961, 134, 214-221.

Bennett, E. L. Brain effects of differential experience and training. In M. R. Rosenzweig & E. L. Bennett (Eds.), Neural mechanisms of learning and memory. Cambridge, Mass.: MIT Press, 1975. In press.

Bennett, E. L., Rosenzweig, M. R., & Wu, S.-Y. C. Excitant and depressant drugs modulate effects of environment on brain weight and cholinesterase. Psychopharmacologia, 1973, 33, 309-328.

Cole, D. D., Sullins, W. R., Jr., & Isaac, W. Pharmacological modification of the effects of spaced occipital ablations. Psychopharmacologia, 1967, 11, 311-316.

Ferchmin, P. A., Bennett, E. L., & Rosenzweig, M. R. Direct contact with enriched environment is required to alter cerebral weights in rats. Journal of Comparative and Physiological Psychology, 1975, 88, 360-367.

Glick, S. D. Change in sensitivity to d-amphetamine in frontal rats as a function of time: Shifting of the dose-response curve. Psychonomic Science, 1970, 19, 57-58.

Glick, S. D., & Zimmerberg, B. Comparative recovery following simultaneous and successive-stage frontal brain damage in mice. Journal of Comparative and Physiological Psychology, 1972, 79, 481-487.

Greenough, W. T., Fass, B., & DeVoogd, T. The influence of experience on recovery following brain damage in rodents: Hypotheses based on development research. In R. N. Walsh and W. T. Greenough (Eds.), Environments as therapy for brain dysfunction. New York: Plenum Press, 1975. In press.

Reference Notes

Note 1. Will, B. E., Rosenzweig, M. R., & Bennett, E. L. Effects of differential environments on recovery from neonatal brain lesions, measured by problem-solving scores. In preparation.

Note 2. Will, B. E., & Rosenzweig, M. R. Effects de l'environnement sur la récuperation fonctionnelle après lésions cérébrales chez des rats adultes. In preparation.

Isaacson, R. L. Experimental brain lesions and memory. In M. R. Rosenzweig & E. L. Bennett (Eds.), Neural mechanisms of learning and memory. Cambridge, Mass.: MIT Press, 1975. In press.

Kircher, K. A., Braun, J. J., Meyer, D. R., & Meyer, P. M. Equivalence of simultaneous and successive neocortical ablations in production of impairments of retention of black-white habits in rats. Journal of Comparative and Physiological Psychology, 1970, 71, 474-480.

Lynch, G. S., Ballantine, P., II, & Campbell, B. A. Potentiation of behavioral arousal after cortical damage and subsequent recovery. Experimental Neurology, 1969, 23, 195-206.

Morimoto, H., Ferchmin, P. A., & Bennett, E. L. Spectrophotometric analysis of RNA and DNA using cetyltrimethylammonium bromide. Analytical Biochemistry, 1974, 62, 436-448.

Petrinovich, L., & Bliss, D. Retention of a learned brightness discrimination following ablations of the occipital cortex in the rat. Journal of Comparative and Physiological Psychology, 1966, 61, 136-183.

Petrinovich, L., & Carew, T. J. Interaction of neocortical lesion size and interoperative experience in retention of a learned brightness discrimination. Journal of Comparative and Physiological Psychology, 1969, 68, 451-454.

Rabinovitch, M. S., & Rosvold, H. E. A closed-field intelligence test for rats. Canadian Journal of Psychology, 1951, 5, 122-128.

Rosenzweig, M. R., & Bennett, E. L. Enriched environments: Facts, factors, and fantasies. In J. L. McGaugh & L. Petrinovich (Eds.), Festschrift for David Krech. New York: Plenum Press, 1975. In press.

Rosenzweig, M. R., & Bennett, E. L. Effects of differential environments on brain weights and enzyme activities in gerbils, rats, and mice. Developmental Psychobiology, 1969, 2, 87-95.

Rosenzweig, M. R., & Bennett, E. L. Cerebral changes in rats exposed individually to an enriched environment. Journal of Comparative and Physiological Psychology, 1972, 80, 304-313.

Rosenzweig, M. R., Krech, D., Bennett, E. L., & Diamond, M. C. Effects of environmental complexity and training on brain chemistry and anatomy: A replication and extension. Journal of Comparative and Physiological Psychology, 1962, 55, 429-437.

Sarno, M. T. Speech therapy and language recovery in severe aphasia. Journal of Speech and Hearing Research, 1970, 13, 607-625.

Schwartz, S. Effect of neonatal cortical lesions and early environmental factors on adult rat behavior. Journal of Comparative and Physiological Psychology, 1964, 57, 72-77.

Smith, C. J. Mass action and early environment in the rat. Journal of Comparative and Physiological Psychology, 1959, 52, 154-156.

Soulairac, A. Modifications de comportement alimentaire du rat à la suite de lésions du cortex cérébral. Journal of Physiology, 1952, 44, 323-326.

Stern, P., McDowell, F., Miller, J. M., & Robinson, M. Factors influencing stroke rehabilitation. Stroke, 1971, 2, 213-218.

Teuber, H.-L. Recovery of function after lesions of the central nervous system: History and prospects. In E. Eidelberg & D. G. Stein (Eds.), Functional recovery after lesions of the nervous system. Neurosciences Research Program Bulletin 12, 1974.

Ward, A. A., Jr., & Kennard, M. A. Effect of cholinergic drugs on recovery of function following lesions of the central nervous system in monkeys. Yale Journal of Biology and Medicine, 1942, 15, 189-228.

Table 1

Mean Initial and Repetitive Errors per Trial, Summed for the 12 Problems; Levels of Significance of Differences between Groups are Shown, Experiment I

	Initial Errors				Repetitive Errors			
A. Trial 1								
Group	EC-S'	IC-S	EC-L	IC-L	EC-S	IC-S	EC-L	IC-L
Mean	25.4	29.9	34.1	33.3	3.5	4.9	13.7	18.2
S.D.	4.0	4.6	4.5	4.3	2.5	3.8	7.7	11.2
EC-S	-	.05[a]	.001	.001	-	NS[a]	.01	.001
IC-S		-	.05	NS		-	.01	.001
EC-L			-	NS			-	NS
B. Trials 2-4								
Mean	14.2	18.3	23.7	30.7	1.3	3.6	3.7	10.3
S.D.	2.4	4.0	4.3	6.8	1.1	2.4	1.8	5.7
EC-S	-	.05	.001	.001	-	NS	NS	.001
IC-S		-	.05	.001			NS	.001
EC-L			-	.01			-	.001
C. Trials 5-8								
Mean	7.5	12.3	16.5	28.6	0.4	1.3	1.6	5.2
S.D.	3.1	4.6	5.3	5.2	0.4	0.8	2.1	2.5
EC-S	-	.05	.001	.001	-	NS	NS	.001
IC-S		-	.05	.001		-	NS	.001
EC-L			-	.001			-	.001

[a] .05 = P <.05; .01 = P <.01; .001 = P <.001; NS = nonsignificant.

Table 2

Mean Total Errors on Trials 2-8, Summed for the 12 Problems; Levels of Significance of Differences between Groups in Experiment II

Group	24h EC-S	2h EC-S	IC-S	24h EC-L	2h EC-L	IC-L
Mean	65.7	59.5	68.2	128.7	114.6	204.6
S.D.	19.0	19.0	21.3	53.5	63.6	113.7
24h EC-S	-	NS[a]	NS	.05	.05	.001
2h EC-S		-	NS	.01	.05	.001
IC-S			-	.05	.05	.001
24h EC-L				-	NS	.01
2h EC-L					-	.001

[a] .05 = P <.05; .01 = P <.01; .001 = P <.001.

Table 3

Mean Initial and Repetitive Errors per Trial, Summed for the 12 Problems; Levels of Significance of Differences between Groups are Shown, Experiment II

	Initial Errors						Repetitive Errors					
A. Trial 1												
Group	24h EC-S	2h EC-S	IC-S	24h EC-L	2h EC-L	IC-L	24h EC-S	2h EC-S	IC-S	24h EC-L	2h EC-L	IC-L
Mean	20.7	22.6	22.3	26.7	24.7	27.4	2.2	4.6	6.4	7.4	7.5	9.0
S.D.	3.5	5.8	6.1	5.7	6.6	9.1	1.5	4.0	4.0	8.1	4.4	6.9
24h EC-S	-	NS[a]	NS	.05	NS	.05	-	NS	.10	.05	.05	.01
2h EC-S		-	NS	NS	NS	.10		-	NS	NS	NS	.05
IC-S			-	NS	NS	.10			-	NS	NS	NS
24h-EC-L				-	NS	NS				-	NS	NS
2h EC-L					-	NS					-	NS
B. Trials 2-4												
Mean	12.0	11.0	11.5	18.4	16.4	23.6	1.4	1.0	1.6	3.8	4.4	12.0
S.D	3.1	2.9	2.9	4.9	4.3	7.5	0.9	0.8	1.4	3.9	3.1	10.7
24h EC-S	-	NS	NS	.001	.05	.001	-	NS	NS	NS	NS	.001
2h EC-S		-	NS	.001	.01	.001		-	NS	NS	NS	.001
IC-S			-	.001	.01	.001			-	NS	NS	.001
24h EC-L				-	NS	.01				-	NS	.001
2h EC-L					-	.001					-	.001

Table 3 (continued)

C. Trials 5-8

Mean	5.7	5.3	6.4	12.5	11.5	18.6	0.5	0.4	0.7	2.9	1.7	5.6
S.D.	2.4	3.0	2.6	5.2	10.4	9.5	0.7	0.6	0.5	3.1	2.1	6.5
24h EC-S	-	NS	NS	.01	.05	.001	-	NS	NS	.10	NS	.001
2h EC-S		-	NS	.05	.05	.001		-	NS	.10	NS	.001
IC-S			-	.05	.05	.001			-	NS	NS	.001
24h EC-L				-	NS	.05				-	NS	.01
2h EC-L					-	.01					-	.05

[a] .10 = $p < .10$; .05 = $p < .05$; .01 = $p < .01$; .001 = $p < .001$; NS = nonsignificant

Table 4

Effects of Lesion and Environment on Brain Weights and Brain RNA/DNA, Experiment II A (N=15 per group)

A. Weight (mg)	EC-S	IC-S	EC-L	IC-L	Percentage Differences: Effect of Environment: EC-S vs. IC-S	Effect of Environment: EC-L vs. IC-L	Effect of Lesion: EC-L vs. EC-S	Effect of Lesion: IC-L vs. IC-S
Cortex								
Occip.(O')[a]	102.8	95.8	71.8	69.8	7.4***	2.9	-30.2****	-27.1****
Somesthetic	58.0	56.7	56.7	55.5	2.2	2.2	-2.1	-2.0
Rem.dorsal	251.6	245.6	236.9	229.3	2.4	3.3	-5.8***	-6.6***
Ventral	276.2	262.3	271.2	263.9	5.3**	2.8	-1.8	0.6
Total (TC)	688.7	660.4	636.7	618.5	4.3***	2.9*	-7.6****	-6.3****
Total-Occip.	585.8	564.6	564.9	548.7	3.8**	2.9	-3.6**	-2.8
Rest of Brain	924.1	937.0	926.0	929.3	-1.4	-0.4	0.2	-0.8
TC/Rest	.745	.705	.688	.666	5.7****	3.2***	-7.7****	-5.5****
(TC-O')/Rest	.634	.603	.610	.591	5.1****	3.2***	-3.8****	-2.0*

Table 4 (continued)

B. RNA/DNA

Cortex								
Occip.(O')	1.606	1.531	1.572	1.483	4.9***	6.0***	-2.2	-3.1*
Somesthetic	1.639	1.601	1.625	1.568	2.4**	2.6****	-0.8	-2.0**
Rem. dorsal	1.730	1.697	1.753	1.722	2.0*	1.8*	1.3	1.5
Ventral	1.548	1.557	1.632	1.617	-0.6	0.9	5.4****	3.9***
Total (TC)	1.628	1.606	1.666	1.630	1.4	2.2**	2.3**	1.5
Total-Occip.	1.633	1.621	1.681	1.654	0.7	1.6*	2.9***	2.0**
Rest of Brain	.597	.604	.600	.608	-1.1	-1.2	0.5	0.6
TC/Rest	2.727	2.659	2.777	2.683	2.5**	3.5***	1.8*	0.9
(TC-O')/Rest	2.735	2.685	2.802	2.722	1.9	2.9**	2.4**	1.4

[a] Enlarged occipital sample; see Figure 6.

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

FIGURE CAPTIONS

Figure 1. Extent of lesions in Experiment I, shown on outlines of the dorsal views of the brains. Lesions were reconstructed from frozen sections. The locations and extents of lesions were similar for the four groups: EC-Drug (ED-D), EC-saline control (EC-C), IC-D, and IC-C.

Figure 2. Coronal sections, illustrating various forms of lesions observed. A. Bilateral cortical lesions that left the corpus callosum and hippocampus unimpaired and undistorted. B. Hippocampus as well as cortex lesioned on right side; hippocampus is distorted and partially fills in the cortical gap on the left side. C. Hippocampus is largely destroyed on right side; distorted hippocampus fills in cortical gap on left. D. Intact but distorted hippocampi fill in sites of cortical lesions in both hemispheres. The width of each block represents 15 mm.

Figure 3. Total errors per rat on trials 2-8, summed over all 12 problems of the Hebb-Williams maze; the vertical lines indicate $\pm$ 1 S.D.

Figure 4. Extent of lesions in Experiment II, shown on outlines of the dorsal views of the brains. Lesions were reconstructed from frozen sections. The locations and extents of the lesions were similar for the three lesioned groups--24-hr EC shown in the top two rows, 2-hr daily EC shown in the center two rows, and IC in the bottom two rows.

Figure 5. Total errors per rat on trials 2-8, summed over all 12 problems of the Hebb-Williams maze, Experiment II. The vertical lines indicate $\pm$ 1 S.D.

Figure 6. Diagram of dorsal view of the rat brain with small plastic T-square employed to demarcate standard samples of cortical tissue. The occipital sample in the present experiment (O') extended further both anteriorly and posteriorly than the sample we typically remove (O).

Figure 7. Effect of a single dose of drug (methamphetamine) on the diurnal cycle of activity. The drug was injected just before 8 o'clock. Each point represents activity averaged over the two hours succeeding the time indicated.

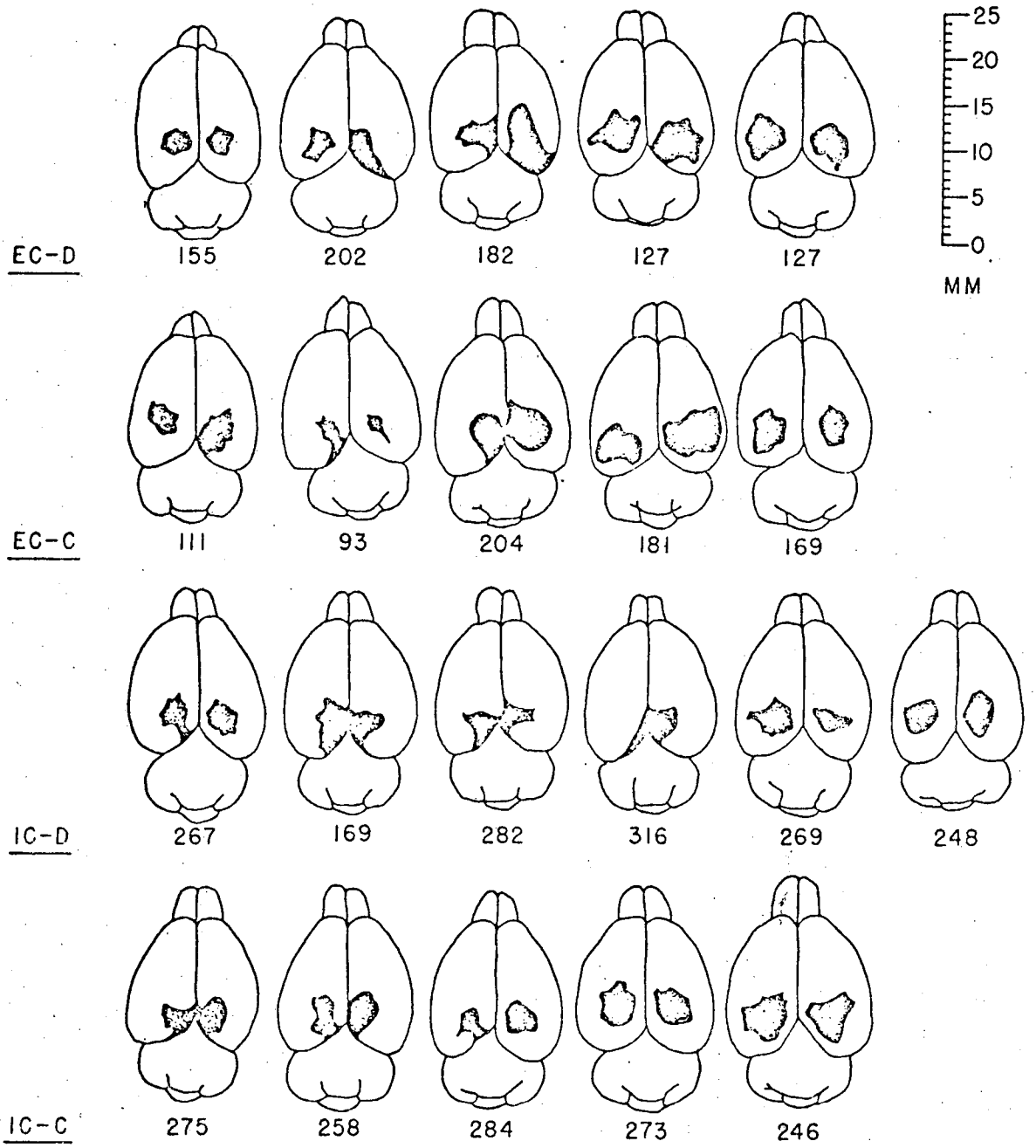


Fig. 1

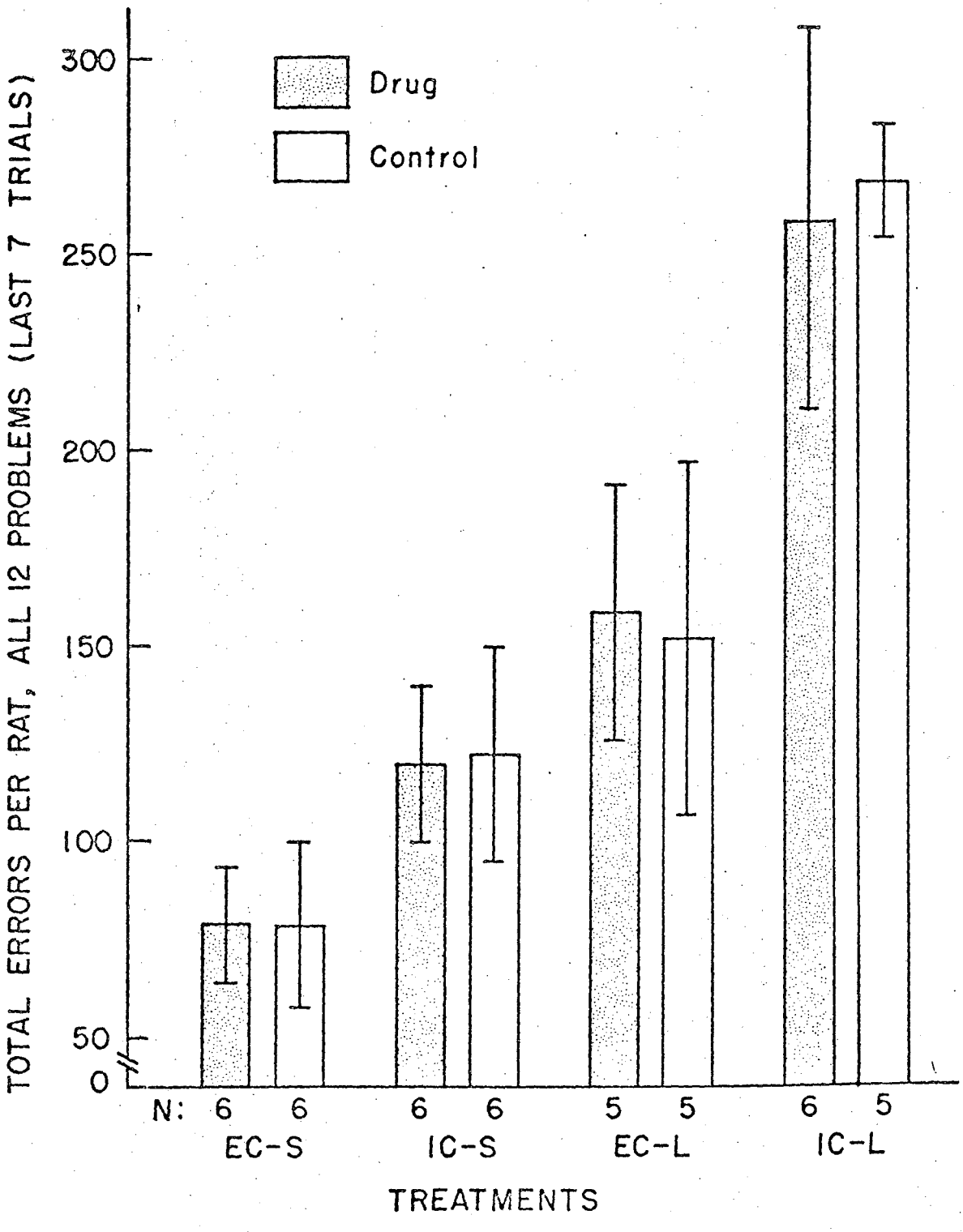

TOTAL ERRORS PER RAT, ALL 12 PROBLEMS (LAST 7 TRIALS)
300
250
200
150
100
50
0
Drug
Control
N: 6 6 6 6 5 5 6 5
EC-S
IC-S
EC-L
IC-L
TREATMENTS
XBL755-5229

Fig. 2

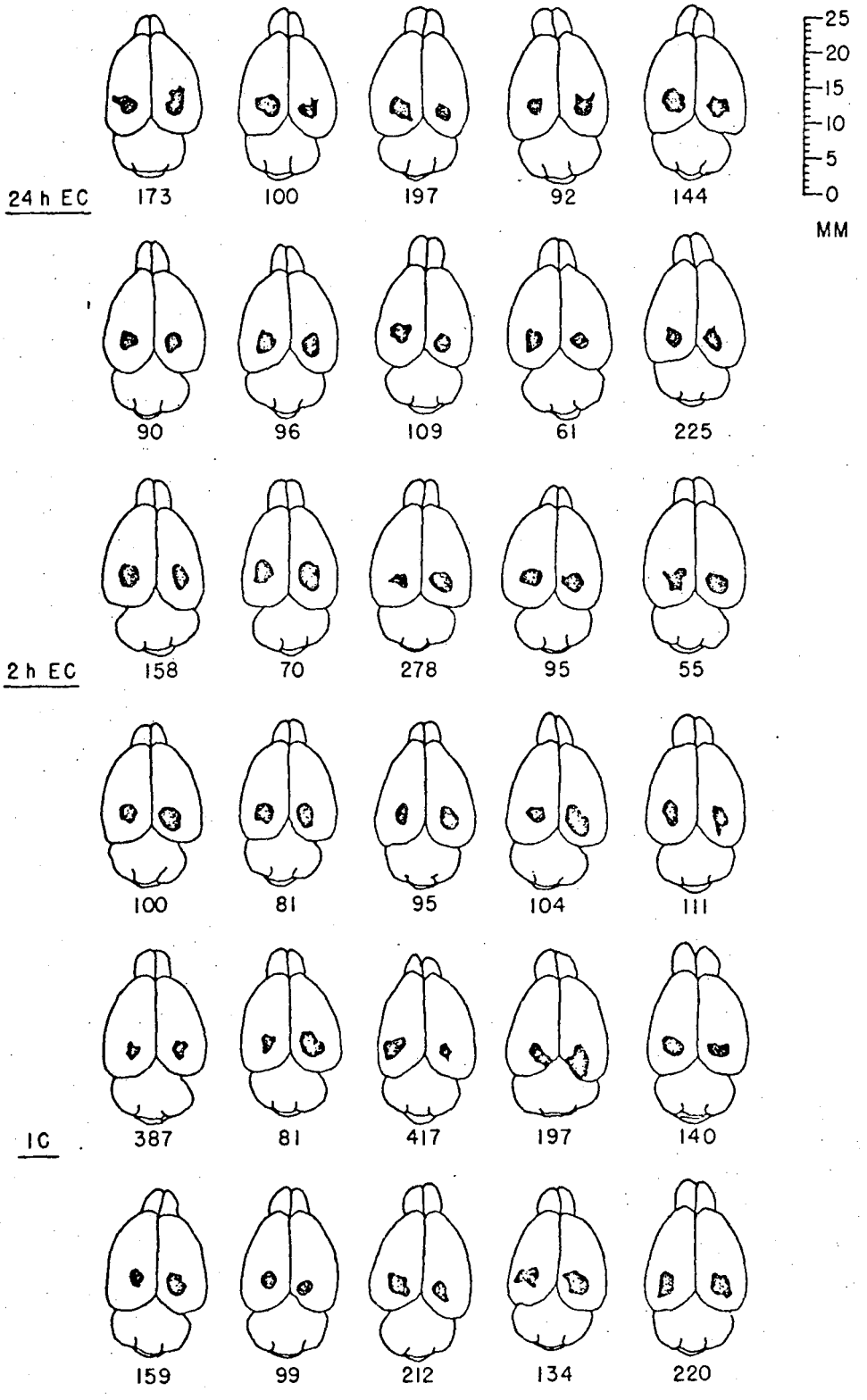


Fig. 3

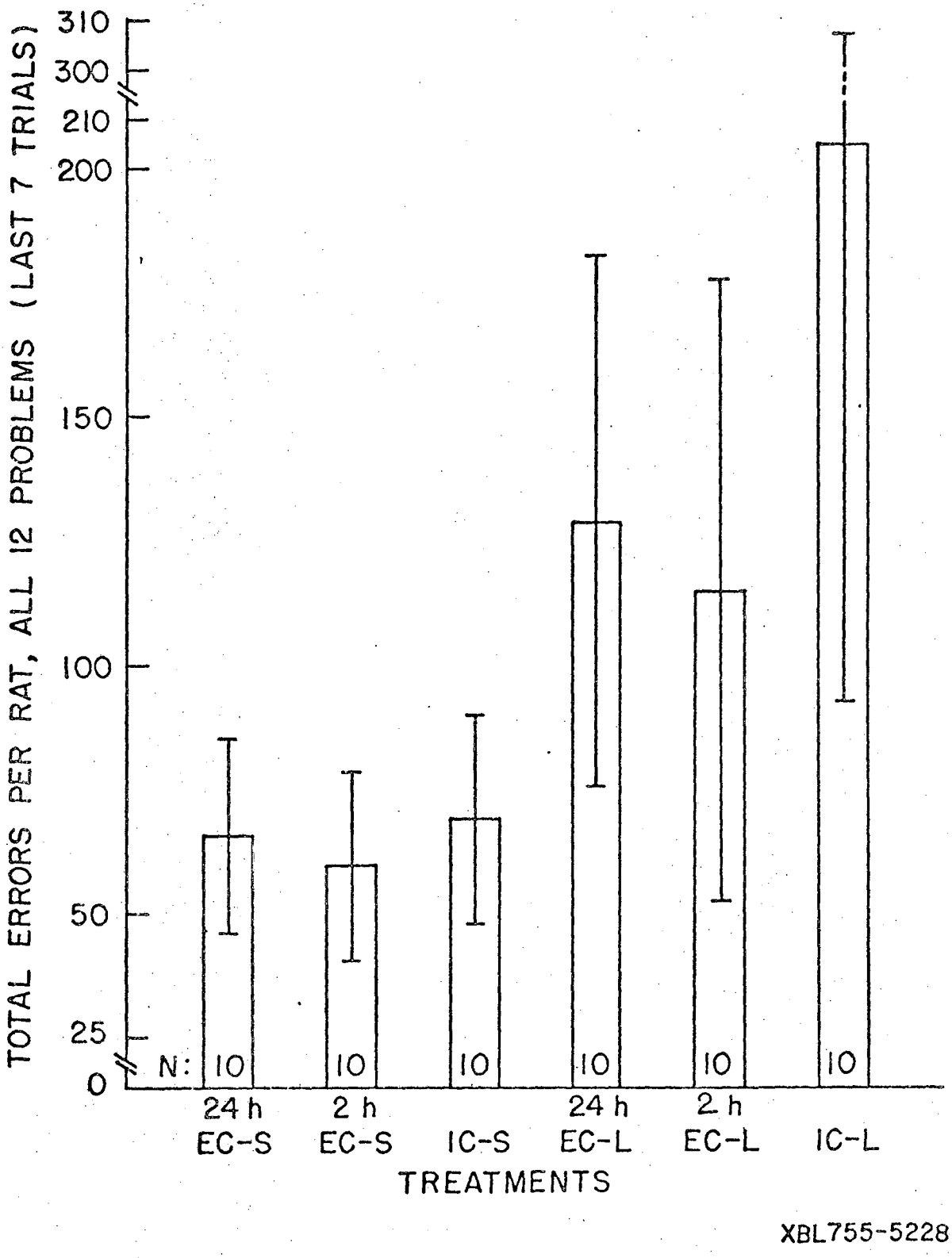
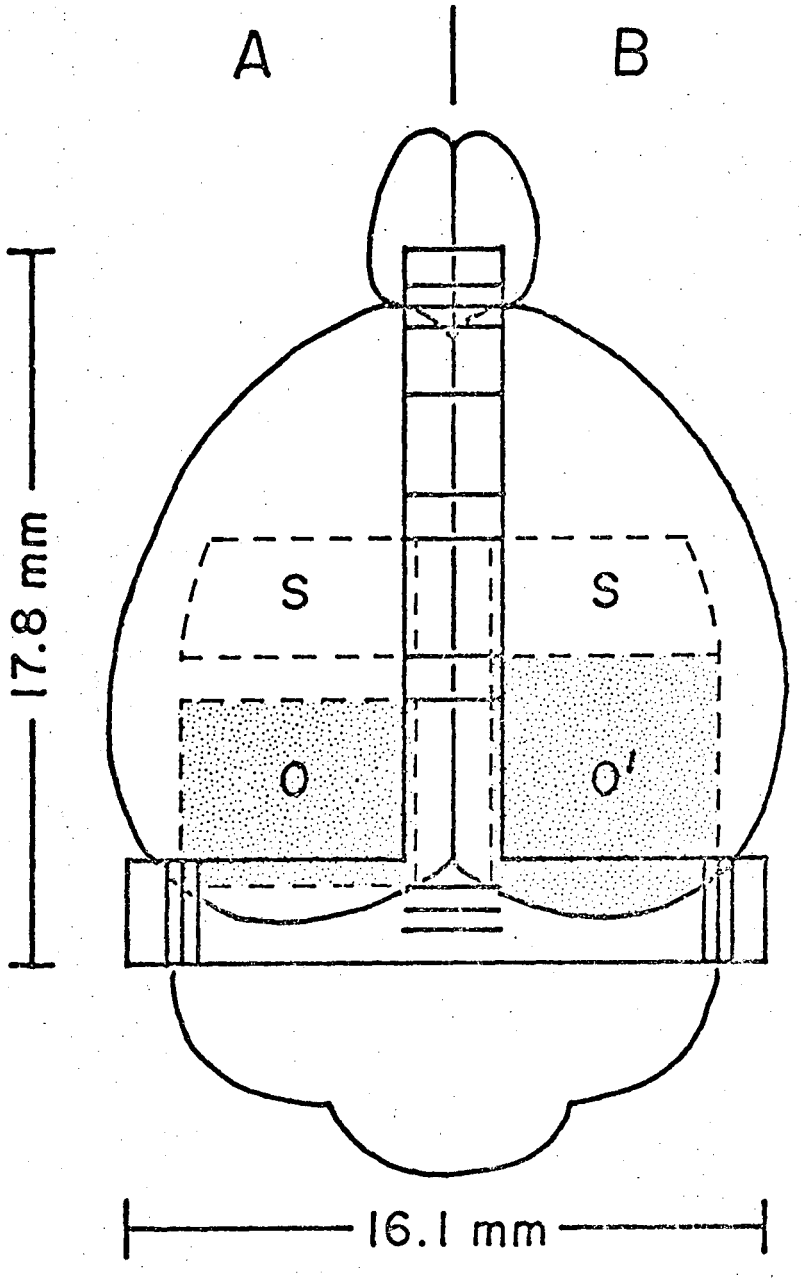

TOTAL ERRORS PER RAT, ALL 12 PROBLEMS (LAST 7 TRIALS)
310
300
210
200
150
100
50
25
0
N: 10 10 10 10 10 10
24 h EC-S
2 h EC-S
IC-S
24 h EC-L
2 h EC-L
IC-L
TREATMENTS
XBL755-5228

Fig. 4



XBL755-5250

Fig. 5

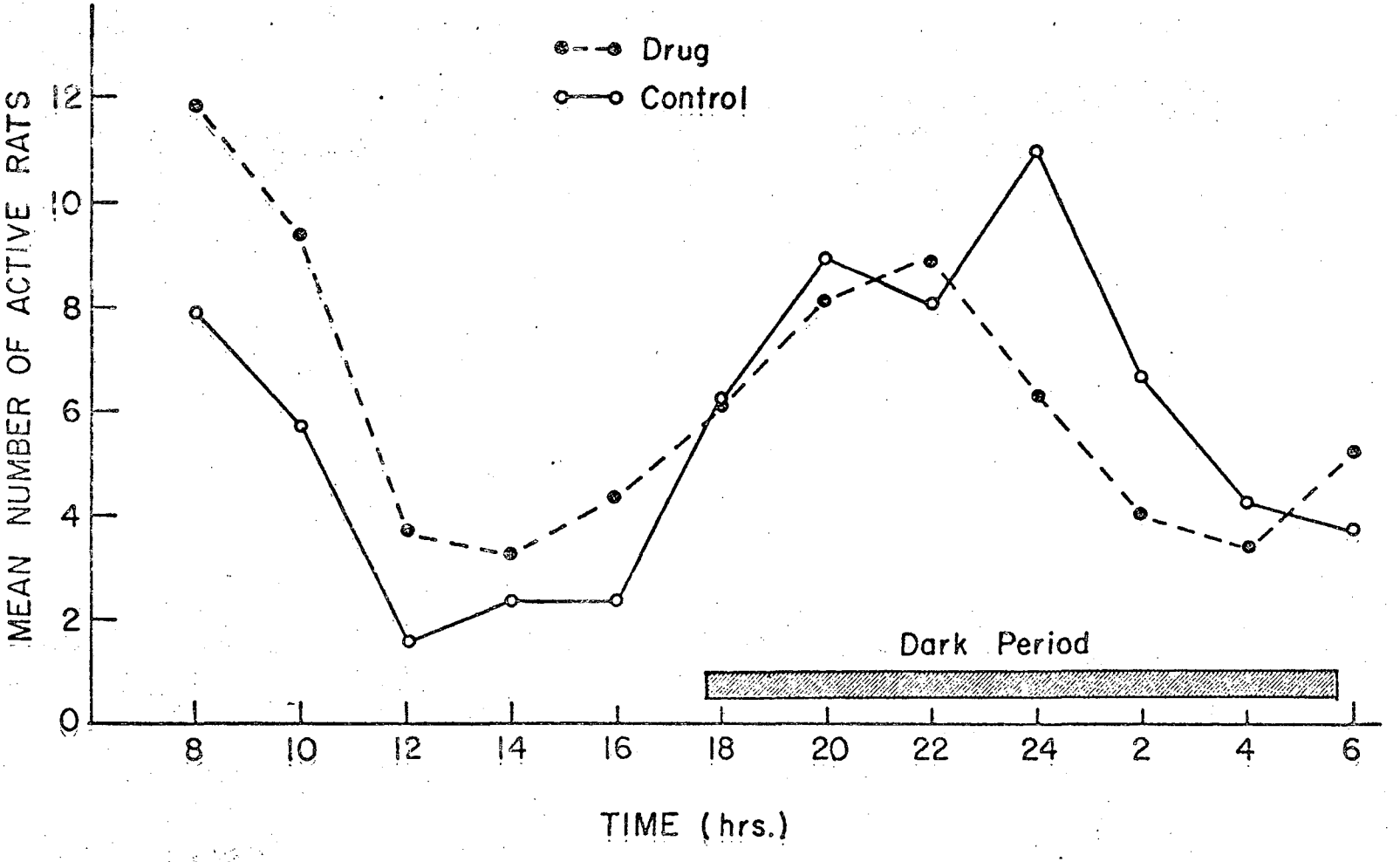

Drug
Control
MEAN NUMBER OF ACTIVE RATS
0
2
4
6
8
10
12
Dark Period
8
10
12
14
16
18
20
22
24
2
4
6
TIME (hrs.)
XBL755-5227

Fig. 6

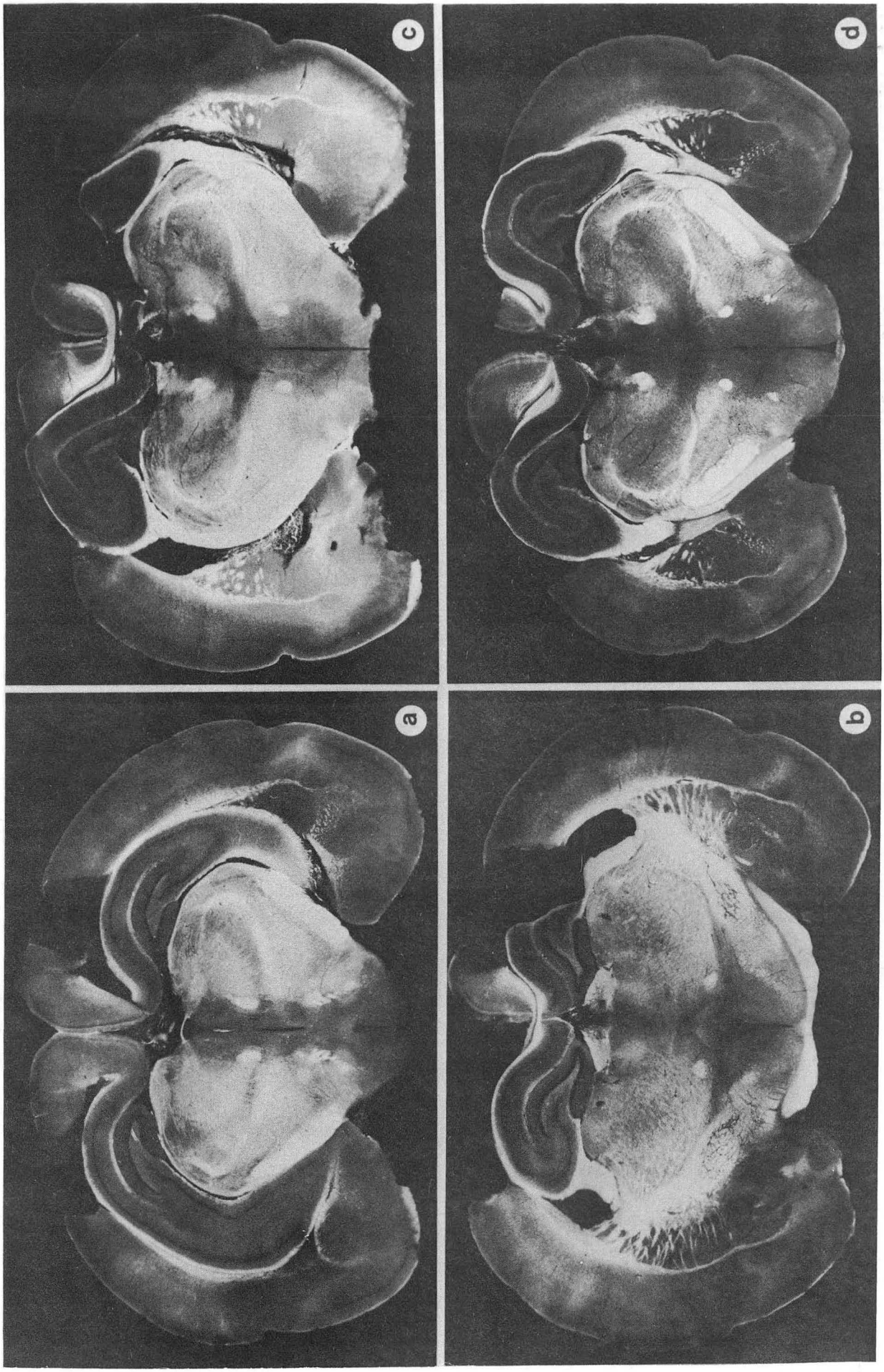


Fig. 7

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LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720