INFLUENCES OF ENVIRONMENTAL COMPLEXITY AND VISUAL STIMULATION ON DEVELOPMENT OF OCCIPITAL CORTEX IN RAT

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INTRODUCTION

The cerebral differences that develop between enriched-experience and impoverished-experience rats appear to be larger in the occipital region of the cortex than in other brain areas, as we have noted previously[2-4]. In this report we will take up three related sets of questions: First, is the larger size of the occipital effect consistent and reliable so that the differences in weight, enzymatic activity and cortical depth are *significantly* greater in the occipital region than in the other brain samples we take? Secondly, how general is this effect? Does the larger magnitude of the occipital effects hold true when we vary age at onset, duration of the experiment, length of treatment per day, strains of rats tested, and species of rodent? When we have established the reliability and generality of the fact that the occipital region of the rat brain is especially modifiable, and knowing that the visual cortex is in the occipital area, we will take as the third problem the role that visual stimulation and experience may play in causing these differences. Thirdly, is it possible that the particular conditions of enriched experience that we have employed are peculiarly visual in nature? Or may the environmental conditions affect the occipital region through non-visual inputs?

(1) MAGNITUDES OF EFFECTS AMONG CORTICAL REGIONS

Methods

The behavioral procedures — which consisted of giving rats a variety of enriched or impoverished environments — will be described separately for each type of experiment. Where the enriched condition included formal training, it will be

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designated as Enriched Condition with Training (ECT); in other cases it will be referred to as Enriched Condition (EC). Littermates were assigned to the groups randomly, with the restriction that littermates differing widely in body weight were excluded and that total body weight of groups was substantially equal; the groups were then assigned randomly to the experimental conditions. The dissection procedure is described and a diagram is given in Rosenzweig *et al.*[10]; a photograph of the dissections appears in Krech *et al.*[8]. For the spectrophotometric analysis of brain acetylcholinesterase (AChE), acetylthiocholine was the substrate and promethezine was used to inhibit cholinesterase (ChE) activity. For analysis of ChE, butyrylthiocholine was the substrate and AChE was inhibited with BW284C51 (1,5-bis-[4-allyldimethylammoniumphenyl]-pentan-3-one diiodide). Analyses were made in duplicate; two AChE values usually agreed within 2% and ChE values, within 3%.

Dissection, weighing and chemical analyses were all done 'blind'; that is, the analysts did not know from what condition any animal came. In those experiments in which some groups were kept in total darkness, both dark-raised and light-raised animals were brought to the laboratory for decapitation in light-proof boxes.

80-day ECT–IC experiments with S_1 strain

Behavioral procedures

Thirteen experiments run from 1960 through 1965 with the Berkeley S_1 strain included groups of male littermates in enriched and impoverished conditions. The animals were assigned to the experimental conditions at weaning (about 25 days of age) and were sacrificed after 80 days (at about 105 days of age). For enrichment or impoverishment, animals were assigned to the ECT or IC conditions described previously[2]. The enriched condition room was brightly lighted from 6 a.m. to 6 p.m.; incident light at the floor level was about 55 ft-candles. The isolation cages were placed in a separate, quiet room that was dimly illuminated (about 1 ft-candle) from 6 a.m. to 6 p.m.

Results

Tissue weights. Table I gives mean weight of the four cortical areas for ECT and IC littermates. The standard deviations given in the table are pooled within-experiment values; that is, they represent variation within but not between experiments. The consistency of differences between ECT and IC littermate animals is shown in section B of the table; this gives the number of pairs in which the ECT animal exceeded its IC littermate. In both the occipital and the remaining dorsal cortex, ECT exceeded IC in three-quarters of the cases. In order to determine the significance of differences between ECT and IC littermates (section C), we employed an analysis of variance that retained differences between littermates while minimizing differences arising among litters. It will be seen that the weight differences between ECT and IC groups are highly significant statistically for each of the 4 cortical regions.

These results are based on wet weight of tissue, but in a recent experiment

TABLE I

WEIGHTS OF FOUR CORTICAL AREAS IN ECT AND IC RATS, ECT–IC PERCENTAGE DIFFERENCES, AND SIGNIFICANCES OF DIFFERENCES AMONG PERCENTAGE OF EFFECTS

Values are based on 13 experiments with S_1 strain; 141 littermate pairs. NS, not significant.

	Cortical areas							
	Occipital		*Remaining dorsal*		*Ventral*		*Somesthetic*	
	$\bar{X}$	*S.D.*	$\bar{X}$	*S.D.*	$\bar{X}$	*S.D.*	$\bar{X}$	*S.D.*
A. Weights (mg)								
ECT	65.0	4.2	283.4	16.6	298.6	23.0	51.4	3.0
IC	61.2	4.6	270.1	15.3	289.0	22.6	50.5	3.1
B. ECT > IC/No. pairs*	106/141		107/141		92/141		84/141	
C. % difference, ECT minus IC**	6.1		4.9		3.3		2.0	
P	< 0.001		< 0.001		< 0.001		< 0.01	
D. Significances of differences:								
Occipital	–		NS		= 0.01		< 0.01	
Rem. dorsal			–		NS		< 0.05	
Ventral					–		NS	

* Number of pairs in which the EC number exceeds the IC member, over total number of pairs.
** Percent difference = 100 (ECT mean minus IC mean)/IC mean.

we have found that the percentage differences between EC and IC are virtually identical whether wet weights or dry weights are used[3]. Furthermore, percentage of protein is the same in ECT and IC brain samples[2]. Therefore, ECT–IC differences cannot be attributed to differences in water content of the brains.

Table I also shows in C that the percentage difference between ECT and IC means was greatest for occipital cortex (6.1%, $P < 0.001$) and least for somesthetic cortex (2.0%, $P < 0.01$). In 12 of the 13 experiments the percentage difference was larger for the occipital than for the somesthetic area. In 11 of the 13 experiments, the occipital percentage effect exceeded those in both remaining dorsal cortex and ventral cortex. Thus the larger magnitude of the occipital effects was highly reproducible.

In order to test whether the effects differed significantly in magnitude among cortical regions, we performed the following analysis: the ratio of the ECT to the IC value was first computed for each cortical area for each pair of rats. The ratios for the 141 pairs were subjected to an analysis of variance, and Duncan's multiple range test was then applied. The results of this analysis are given in part D of Table I. The size of the occipital effect is seen to be significantly greater than those in both the somesthetic and the ventral areas. The percentage change in remaining dorsal cortex significantly exceeded that in the somesthetic area. The complex environment thus has more specific effects than simply promoting growth of cortex in general, and it produces its largest effects in the occipital area.

TABLE II

PERCENTAGE DIFFERENCES IN ACETYLCHOLINESTERASE ACTIVITY BETWEEN ECT AND IC GROUPS, IN FOUR CORTICAL AREAS

Values are based on 13 experiments with S_1 strain; 141 littermate pairs.

	Cortical areas			
	Occipital	*Remaining dorsal*	*Ventral*	*Somesthetic*
Total AChE				
% difference, ECT minus IC	2.3	2.4	1.6	0.4
ECT > IC/No. pairs	83/140	86/140	76/140	67/141
P	< 0.01	< 0.001	< 0.10	NS
AChE/weight				
% difference, ECT minus IC	−3.6	−2.2	−1.8	−1.6
ECT > IC/No. pairs	37/140	42/140	56/140	56/141
P	< 0.001	< 0.001	< 0.05	< 0.001

Acetylcholinesterase activity. When AChE activity is compared between ECT and IC animals, it is found that total AChE activity is significantly greater in the ECTs in occipital and remaining dorsal cortex (Table II). The percentage differences are not large, and only the consistency of effects in these 2 regions allowed the finding of significance. The differences in magnitude of effects in total AChE among regions did not reach statistical significance. AChE activity per unit of weight (AChE/weight) is significantly lower in ECT than in IC animals. The decrease in AChE/weight is shown in Table II to be greatest in occipital cortex and least in somesthetic cortex. Here an analysis of variance demonstrated the occipital effect to exceed significantly the effects in the other 3 areas. The effects did not differ significantly among the other areas. Thus, in changes in AChE activity per unit of weight, the occipital area stands out clearly from the rest of the cortex.

Cholinesterase activity. The less specific enzyme, ChE, has been analyzed in the last 9 of the 13 S_1 ECT-IC experiments. For the 98 littermate pairs, the ECT–IC difference in total activity of ChE was greatest in the occipital cortex (10.2%, $P < 0.001$) and least in the somesthetic cortex (4.7%, $P < 0.001$). The occipital effect was larger than the somesthetic effect in 7 of the 9 experiments but, partly because the ChE effects are rather variable in magnitude, the differences among regions were not statistically significant.

ChE activity per unit of weight, unlike AChE/weight, was greater in the ECT than in the IC animals. In ChE/weight, ECT exceeded IC by 4.7% ($P < 0.001$) in the occipital area and by 3.4% ($P < 0.001$) in the somesthetic area. ChE/weight failed to show the significant differences among cortical areas that occurred for both tissue weight and AChE/weight.

Cholinesterase activity/acetylcholinesterase activity. ChE is relatively rich in glial cells, while AChE is found especially in neurons. Since glial proliferation occurs

TABLE III

PERCENTAGE DIFFERENCES IN CORTICAL DEPTHS OF EC AND IC RATS IN OCCIPITAL AND SOMESTHETIC AREAS

Values are based on 4 experiments with S_1 strain; 39 littermate pairs.

Segment	*Occipital*		*Somesthetic*		*Significance of Occip.–Somes. difference*
	%	*P*	%	*P*	
B (dorsal)	6.8	< 0.001	5.0	< 0.001	< 0.01
C (dorsolateral)	7.2	< 0.001	3.7	< 0.001	< 0.001
D (lateral)	6.1	< 0.001	2.6	< 0.01	< 0.01

in rats given complex experience[1,5], changes in the ratio of ChE to AChE may reflect altered glial number and function. In addition, the ChE/AChE ratio provides a purely chemical ratio in which weight of the tissue samples is eliminated. This ratio measure is available for the last 9 of the 13 S_1 ECT–IC experiments. The percentage ECT–IC differences by cortical area were as follows: occipital, 8.6% ($P < 0.001$); ventral, 5.7% ($P < 0.001$); remaining dorsal, 5.6% ($P < 0.001$); and somesthetic, 5.0% ($P < 0.001$). An analysis of variance demonstrated that the effect on this chemical measure was significantly larger in the occipital region than in either the somesthetic area or the remaining dorsal cortex.

In order to economize space, most enzymatic measures in the rest of this article will be given only in terms of activity per unit of weight, since this measure is used more frequently than is total enzymatic activity.

Cortical depths. In a previous study[4], we reported that the depth of cortex was significantly greater in ECT than in IC rats in the occipital region, whereas other cortical regions did not show significant differences. In that study there were too few cases where both occipital and somesthetic measures were made in the same animals to allow critical tests of regional differences in magnitude of the depth effect. We have recently completed a series of 4 experiments measuring the dorsal cortical depths of S_1 rats exposed to EC or IC conditions from 60 to 90 days of age ($N =$ 39 pairs). (As will be shown in the next section, the 60–90-day period yields cortical weight differences that are larger in the occipital than in the somesthetic region.)

The depth of the cortex was determined from enlarged transverse histological sections of the rat brain, as described in the previous study[4]. Each hemisphere was divided into segments B (dorsal), C (dorsolateral) and D (lateral), as shown in Fig. 2 of Diamond[4].

Table III shows that in each segment of the occipital area, there was a highly significant depth difference in favor of the EC rats. The somesthetic area also showed significant EC–IC differences. For each segment, the occipital difference was significantly greater than the somesthetic difference (see the right-hand column of the table). These histological measurements, as well as the weight and chemical analyses, demonstrate that the occipital area of rat cortex is more plastic than the somesthetic area.

(2) GENERALITY OF LARGER OCCIPITAL EFFECTS

In further experiments we have varied both the age at which the animals were exposed to the differential conditions and the duration of the experiments. Strains other than the S_1 line have also been used. Length of exposure to the complex environment has been decreased in some experiments to 2 h a day. In all of these variants, the occipital area has yielded larger differences between enriched- and impoverished-experience animals, as the next sections will demonstrate.

Age. In four experiments run from 1962 to 1966, animals were assigned to ECT or IC conditions at 105 days of age and kept there until 185 days of age. These rats were therefore given their differential experience as adults, since they become sexually mature at about 60 days and since rapid early development of the brain has tapered off around 60–80 days. The mean percentage ECT–IC difference in weight of occipital cortex was 10.9% ($P < 0.001$), while that for the somesthetic area was only 2.8% ($P < 0.05$). Here, as for the younger animals, the ECT–IC difference was about 3 times as great in the occipital as in the somesthetic region, and the occipital difference exceeded the somesthetic difference in each of the 4 experiments. In enzymatic activity per unit of weight, the occipital changes in the adult rats were also significant and exceeded those in the somesthetic area. Thus the brain of the rat, and especially the occipital region, remains plastic to effects of experience after the period of rapid early development.

30-Day EC–IC experiments. Beginning in 1966 we have run a number of experiments in which exposure to the differential environments was limited to 30 days. No formal training was given, so the enriched condition will be referred to as EC rather than ECT. Cortical weight differences between EC and IC groups were actually larger than those found between ECT and IC in the 80-day experiments.

In 10 experiments involving 111 S_1 littermate pairs, the rats were assigned to the experimental conditions at about 25 days of age and were removed at about 55 days of age. In all 10 experiments, the occipital percentage difference exceeded the somesthetic difference. The mean EC–IC difference in weight of occipital cortex was 10.4% ($P < 0.001$), while for somesthetic cortex it was 5.0% ($P < 0.001$). AChE/weight and ChE/weight were determined in 5 experiments with 61 littermate pairs. Occipital EC–IC differences were highly significant and were clearly larger than the somesthetic differences.

In six experiments with 63 S_1 littermate pairs, the animals were held under colony conditions until about 60 days of age when they were assigned to the EC or IC conditions for 30 days. In 5 out of 6 experiments, the occipital weight difference surpassed the somesthetic effect. The overall mean EC–IC weight effect was 8.0% ($P < 0.001$) in occipital cortex and 5.2% ($P < 0.001$) in the somesthetic area. The chemical effects in the 60–90-day experiments did not reveal regional differences as clearly as did the weight effects. In AChE/weight, both the occipital and the somesthetic effects were significant, and both were similar in magnitude. In ChE/weight, neither area showed a significant EC–IC difference.

We conclude that on most measures the 30-day as well as the 80-day experiments reveal the greater modifiability of the occipital area.

Brief daily EC. In several recent experiments, animals were placed in EC for only 2 or 4 h per day; the rest of the day was spent in either the colony condition (3 rats to a cage) or in individual cages. Both S_3 and S_1 rats were used. 2 h of EC per day was found to produce as large cerebral differences from IC as did 24 h of EC per day. In all 2-h EC experiments already reported[11] and in two subsequent 2-h EC experiments, the EC–IC percentage differences in cortical weight and in enzymatic activity were larger in the occipital cortex than in the somesthetic area.

Strain. In order to rule out the possibility that the greater modifiability of the occipital region might be peculiar to the Berkeley S_1 strain of rats, experiments were conducted with other strains. Four experiments were run with 44 littermate pairs of the Berkeley S_3 line. These experiments duplicated the conditions of the S_1 ECT–IC experiments that lasted from 25 to 105 days of age, and they were run concurrently with some of the S_1 studies.

The S_3 animals yielded slightly smaller ECT–IC weight differences than the S_1's, but again the mean effects were clearly larger in the occipital region (5.0%, $P < 0.001$) than in the somesthetic region (0.4%, not significant (NS)). In AChE/weight, the decline was greater in the occipital area (—2.3%, $P < 0.05$) than in the somesthetic area (—0.9%, NS), just as it had been for the S_1 strain. In ChE/weight, the positive ECT–IC effect was greater in the occipital area (7.3%, $P < 0.001$) than in the somesthetic area (2.9%, $P < 0.01$), again following the pattern observed in the S_1 strain. And the ratio of ChE to AChE also followed the S_1 pattern, the ECT–IC difference being 10.6% ($P < 0.001$) in the occipital area as against only 4.0% ($P < 0.01$) in the somesthetic area.

Since the S_1 and S_3 strains were derived from a common foundation stock, the similarity of the distribution of effects within their cortices may not be sufficient evidence of the generality of predominance of the occipital effect. Therefore, some observations have been made on other strains. For example, one experiment was done with 12 littermate pairs of the Long–Evans strain. The ECT–IC effect in weight of occipital cortex (3.9%, $P < 0.10$) was greater than the somesthetic effect (1.4%, NS), but smaller than the difference in remaining dorsal cortex (6.0%, $P < 0.05$). In both AChE/weight and ChE/weight, the occipital effects were greater than those in any other cortical section. Further evidence comes from a recent experiment that included 12 littermate pairs of the inbred Fischer strain, run in EC or IC from 25 to 55 days of age. Here the EC–IC difference in weight was clearly larger in the occipital region (9.3%, $P < 0.001$) than in the somesthetic region (—3.2%, NS) or in any other region. The chemical results also followed the expected pattern: the negative EC–IC difference in AChE/weight was greater in the occipital area than in any other area, and the positive effect in ChE/weight in the occipital area exceeded that in all but the ventral cortical area; the effect in ratio of ChE to AChE was clearly largest in the occipital area. We conclude that the greater modifiability of the occipital area is likely to be found generally among strains of rats.

Species of rodent. We have also asked whether the larger size of the occipital effects will be found among other species. While one inbred strain of mice (A/Crgl) did show the largest ECT–IC weight effects in the occipital area, another inbred mouse

strain (C57BL/Crgl) showed the largest effects in the ventral and somesthetic areas[9]. Recently we have run two experiments with gerbils (*Meriones unguiculatus*) in EC or IC from 30 to 60 days of age. The percentage EC–IC weight differences did not differ clearly among dorsal cortical regions, being 4–6% in occipital, somesthetic, motor, and remaining dorsal cortex. In the enzymatic measures the gerbil occipital region did show larger effects than did other regions. Thus the special plasticity of the occipital area may be less consistent in other rodents than in the rat and should not be extrapolated even to other rodents without direct experimental evidence.

(3) INFLUENCES OF VISUAL STIMULATION

Having seen that our enriched condition consistently affects the rat's occipital region more strongly than the other cortical regions studied, let us now consider the possible role of visual experience in determining these cerebral effects. Studies directed to this problem have employed a variety of means — eliminating visual experience, varying ambient illumination, and enhancing the salience of visual stimuli. We will take up in turn effects of these treatments:

(a) Complete deprivation of visual experience through blinding.
(b) Almost complete deprivation of visual experience through dark-raising.
(c) Varied levels of ambient illumination.
(d) Intermittent visual stimulation with no contingent reward.
(e) Intermittent visual stimulation with contingent reward.

Effects of blinding

We reported in 1963 (ref. 8) that even blinded rats developed rather typical cerebral ECT–IC differences; these experiments lasted from 25 to 105 days of age. One of the experiments reported at that time allowed direct comparison between ECT–IC effects in sighted and blinded animals, because each of eleven S_1 litters furnished an animal to each of the following four groups: sighted ECT (S–ECT), blinded ECT (B–ECT), sighted IC (S–IC) and blinded IC (B–IC). Data of a hitherto unpublished replication of this experiment with 9 S_1 litters have now been combined with those of the original sighted–blinded ECT–IC experiment. Table IV presents some of the principal results.

For weight of occipital cortex (top row of Table IV), there were significant ECT–IC differences among the blinded as well as among the sighted animals. Furthermore, although the effect among the blinded animals (3.6%, $P < 0.05$) was somewhat smaller than among the sighted (5.9%, $P < 0.01$), the difference between these two percentages was not significant, as is shown by the lack of significant interaction between the ECT–IC and S–B variables. For weight of total cortex as well, both blinded and sighted rats showed significant ECT–IC effects, with no significant difference between the magnitude of the two effects. It should also be noted that even among the blinded rats, the percentage effect is larger in occipital cortex than in total cortex. The overall difference between all ECT and all IC animals, combining sighted and blinded, is given to the right of the table under the heading 'Percent Differences, ECT *vs.* IC'.

TABLE IV

EFFECTS OF ENVIRONMENTAL CONDITIONS (ECT *vs.* IC) AND PRESENCE OR ABSENCE OF VISION (S *vs.* B) ON WEIGHT AND ACETYLCHOLINESTERASE ACTIVITY IN CORTEX

Two S_1 experiments combined; $N = 20$ for each of the 4 groups.

	Weight (mg)						
	Sighted		*Blinded*		*Percent differences*		*Interaction ECT–IC vs. S–B*
	ECT	*IC*	*ECT*	*IC*	*ECT vs. IC*	*S vs. B*	
Occipital cortex $\bar{X}$	64.9	61.3	62.9	60.7	4.8§	2.1	NS
% diff.	5.9***		3.6**				
Total cortex $\bar{X}$	694	674	684	662	3.1§	1.6*	NS
% diff.	3.0***		3.2***				
	AChE/weight§§						
Occipital cortex $\bar{X}$	5.58	5.67	6.01	6.15	−2.0*	−7.4§	NS
% diff.	−1.5		2.4*				
Total cortex $\bar{X}$	8.40	8.43	8.48	8.56	−0.6	−1.2	NS
% diff.	−0.3		−0.8				

* $P < 0.10$.
** $P < 0.05$.
*** $P < 0.01$.
§ $P < 0.001$.
§§ Acetylcholinesterase activity is expressed in units of μmoles acetylthiocholine hydrolyzed/min/mg.

The ECT–IC weight difference was significant for both occipital and total cortex. On the other hand, the overall difference between all sighted and all blinded animals, combining ECT and IC, was not significant for occipital cortex and reached only the 0.10 level of significance for total cortex.

In AChE activity — shown in the lower half of Table IV — the only ECT–IC difference to approach significance was that among blinded rats in occipital cortex. Sighted animals showed a highly significant decrease in AChE/weight in occipital cortex but none in total cortex. Although sighted had slightly greater weight of occipital cortex than did blinded, the relatively large drop in AChE/weight brought total AChE activity in occipital cortex down 5.6% ($P < 0.01$) below that of the blinded rats.

It is clear that the pattern of differences induced in the occipital cortex by sight *vs.* blinding (Table IV) is dissimilar from the pattern induced by environmental enrichment *vs.* deprivation (Tables I and II). These patterns of effects will be considered further in the discussion.

Effects of dark-raising

Blinding by enucleation in the rat results in prompt degeneration of primary

TABLE V

COMPARISONS BETWEEN ECT LITTERMATES KEPT UNDER NORMAL LIGHT CYCLE (L) OR IN COMPLETE DARKNESS (D)

Three S_1 experiments combined: $N = 31$ per group.

	Weight (mg)	*AChE*	*ChE*
		wt	*wt*
Occipital cortex			
L	64.6	5.5	0.385
D	60.1	6.0	0.372
% diff.§	7.4***	−9.7***	3.5**
Total cortex			
L	698	8.4	0.367
D	687	8.6	0.364
% diff.§	1.6*	−2.6**	1.0

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
§ 100 (Light minus Dark)/Dark.

visual pathways through the lateral geniculate body; it also produces less dramatic changes in the cortical visual centers. The blinded animal therefore suffers anatomical loss in addition to deprivation of visual stimulation. To determine effects when only sensory deprivation was involved, we have conducted several experiments in which sighted littermate animals were raised either under a normal light-cycle or in total darkness.

80-Day ECT in light or dark

In 3 experiments S_1 rats were kept from 25 to 105 days of age under standard ECT conditions, either under a normal light-cycle or in total darkness. The dark-ECT condition included open field exploration and maze training, although visual cues were of course excluded. Prior tests showed that rats could discriminate visual form even under a red photographic safelight, so absolute darkness was maintained. These experiments included 31 littermate pairs.

Results. Some of the principal results for light- *vs.* dark-raising on occipital and total cortex are given in Table V. It can be seen that the animals with visual experience developed significantly greater weight of occipital cortex than did littermates kept in the dark. None of the other three cortical samples showed a significant light–dark effect, so they are not included in the table. Total cortex was significantly heavier in the light-raised animals, but about half of this effect was due to the occipital sample which accounted for less than one-tenth of the weight of total cortex.

AChE activity per unit of weight was significantly greater in both the occipital and total cortex of the dark-raised rats than in their light-raised littermates. This is similar to the increase of AChE/weight above normal levels found in the blinded rats

TABLE VI

EFFECTS OF ENVIRONMENTAL CONDITIONS (EC *vs.* IC) ON BRAIN WEIGHTS OF RATS UNDER NORMAL LIGHT CYCLE OR IN DARKNESS

Three S_1 experiments combined; $N = 23$ light pairs, $N = 22$ dark pairs.

	Normal light cycle				*Dark-raised*				
	EC $\bar{X}$	*IC* $\bar{X}$	*% diff. EC–IC*	*P*	*EC* $\bar{X}$	*IC* $\bar{X}$	*% diff. EC–IC*	*P*	*% diff. L–EC minus D–IC*
Occip. cortex	58.6	54.6	7.3	< 0.01	55.2	51.0	8.2	< 0.001	14.9
Total cortex	662	629	5.2	< 0.001	646	612	5.5	< 0.001	8.1
Rest of brain	817	808	1.0	NS	814	789	3.1	< 0.01	3.5
Total brain	1479	1438	2.9	< 0.01	1460	1401	4.2	< 0.001	5.5

in the previous section. ChE/weight of occipital cortex was significantly greater in the light-raised than in dark-raised rats.

Thus in animals having normal vision and exposed to a complex environment, the presence of light clearly affects development of the brain, and especially of the occipital region of the cortex. Furthermore, the light–dark differences in these experiments are similar in both weight of cortex and ChE activity to the ECT–IC differences that we reported above. However, the light–dark difference in AChE/weight is considerably greater than the corresponding ECT–IC effect and resembles the sighted–blind effect.

30-Day EC–IC in light or dark

While the preceding experiments studied effect of light or dark only on ECT animals, 3 further experiments included both EC and IC groups in both light and dark conditions. The litters for the dark conditions were kept in total darkness either from birth or from 10 days (before their eyes had opened). Weaning of the dark-raised animals was done under a red photographic safelight when the animals were about 25 days of age. On the day of weaning, pairs from the light-raised and dark-raised litters were assigned to EC or IC cages, remaining under the condition of illumination in which they had been up to weaning. The EC animals lived in large cages equipped with toys, but they had neither open-field experience nor maze training. There were thus littermates in EC and IC conditions under a normal light-cycle (L–EC and L–IC) and, from other litters, EC and IC littermates kept in the dark (D–EC and D–IC). (Because of this design, critical littermate comparisons can be made here between L–EC and L–IC and between D–EC and D–IC but not between L and D animals.) All subjects were of the S_1 strain and, except in the second of the 3 experiments, they were males.

During the experiment photographic film was placed in the dark room to monitor possible light leaks. There were occasional leaks, most likely when the door to the outer vestibule was opened, but this probably amounted to only a minute or two in the course of an entire 30-day experiment.

Results. Weights and weight differences are given in Table VI. The overall EC–IC difference in weight of the occipital cortex was 7.3% ($P < 0.01$) for the light-raised ($N = 23$ pairs) and surprisingly it was slightly greater — 8.2% ($P < 0.001$) — for the dark-raised animals ($N = 22$ pairs). Both light-raised and dark-raised yielded significant EC–IC differences in weights of the other 3 cortical regions as well, but none of the other regions gave as large percentage differences as did the occipital cortex.

It is possible to estimate the combined effects of EC–IC and light–dark conditions if we are willing to compare non-littermate L–EC with D–IC animals. These percentage differences are given in the last column of Table VI. At occipital cortex, the combined effects yield a difference of 14.9%; for total cortex the difference is 8.1%. Both treatments affect chiefly the cortex, for in the rest of the brain the difference between L–EC and D–IC amounts to only 3.5%.

In AChE/weight of occipital cortex, the EC–IC difference reached —4.1%

($P < 0.001$) among the light-raised and —3.9% ($P < 0.001$) among the dark-raised. Here again there were also significant differences in total cortex. In ChE/weight of occipital cortex, the light-raised showed a significant effect (3.9%, $P < 0.01$), but the dark-raised did not (0.2%, NS).

It should be noted that the experiment with female subjects yielded results similar to those with males; specifically, the occipital cortex again showed the largest differences. A further light–dark experiment with female S_1 rats, not otherwise mentioned in this paper, also replicated results obtained with males. Thus the generality of findings extends across sex as well as across strain and age of subjects.

The results of the light–dark EC–IC experiments, as well as those in the earlier sighted–blinded experiments, demonstrate that significant differences develop in the occipital cortex as a function of enriched experience, whether or not vision plays a part in that experience. Furthermore, the cerebral effects of differential environmental complexity (EC *vs.* IC) are fully as large as those produced by normal light-cycle *vs.* dark-raising. Not only is it clear that visual experience is not necessary for the development of the EC–IC effects, but also we find that the EC–IC effects do not simply duplicate those involving presence or absence of the visual receptors or of visual experience.

Effects of varied levels of illumination

If effects in occipital cortex can be determined through other than visual channels, it is still necessary to inquire whether different amounts or types of visual experience can be demonstrated to alter brain measures. That is, under normal conditions of vision, is variation in visual experience a factor in determining the cerebral differences even if it is not a necessary cause?

One may ask whether the difference in illumination between the EC room (about 55 ft-candles) and the isolation room (about 1 ft-candle) might by itself account in large part for the usual cerebral differences found as a result of the EC–IC environments. (The illumination in the isolation room is sufficient for the caretaker to weigh the animals and to record their weights easily.) In a few experiments we have therefore varied these conditions, putting an EC cage in the dimly illuminated IC room, or isolating animals in the large brightly lighted laboratory where the EC cages are kept.

Effects of EC under dim illumination

An experiment testing this point included 4 male S_1 rats from each of 11 litters, assigned to these conditions: (a) Standard ECT condition. (b) Environmental Complexity (EC); the animals lived in the large cage with toys but they received no maze training. (c) Environmental Complexity in Isolation Room (ECI). This was the same as condition b except that the large cage was placed in the isolation room along with the IC animals. (d) Standard IC.

Results. When the results for weight and enzymatic activity of the occipital cortex are considered (Table VII), it is apparent that the Environmental-Complexity-in-Isolation group (which shared the dimly illuminated room with the IC group)

TABLE VII

EFFECTS OF ENVIRONMENTAL CONDITIONS ON WEIGHT AND ACETYLCHOLINESTERASE ACTIVITY OF CORTEX

S_1 strain; $N = 11$ per group.

	Weight						
	Means (mg)				*Percentage diffs. from IC§*		
	ECT	*EC*	*ECI§§*	*IC*	*ECT*	*EC*	*ECI*
Occip. cortex	64.0	62.0	64.7	60.2	6.3*	3.0	7.4*
Total cortex	706	696	686	672	5.1**	3.6*	2.0
	AChE/weight						
Occip. cortex	5.1	5.3	5.4	5.6	−8.7***	−5.4*	−4.2
Total cortex	8.2	8.5	8.5	8.8	−6.7***	−3.9*	−3.5*

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
§ 100 (Enriched minus IC)/IC.
§§ Environmental Complexity in Isolation Room.

TABLE VIII

COMPARISONS IN BRAIN MEASURES BETWEEN AN ENRICHED-EXPERIENCE GROUP, TWO ISOLATED GROUPS IN BRIGHT ILLUMINATION, AND AN ISOLATED GROUP IN DIM ILLUMINATION

S_1 strain; $N = 12$ per group.

Illumination	*Bright*	*Bright*	*Bright*	*Dim*	*Percentage differences between ECT and respective IC groups*		
Condition	*ECT*	*IC-(A + H)§*	*IC(C)§§*	*IC*	*IC-(A + H)§*	*IC(C)§§*	*IC*
Weight (mg)							
Occipital cortex	72.8	67.9	68.5	68.2	7.2*	6.3*	6.7*
Total cortex	690	667	654	664	3.5*	5.6**	4.0*
AChE/weight§							
Occipital cortex	6.1	6.4	6.7	6.4	−5.0*	−8.6***	−5.2*
Total cortex	9.1	9.2	9.6	9.3	−1.4	−5.0**	−2.3

* $P < 0.05$.
** $P < 0.01$.
*** P 0.001.
§ Animals in isolation cages, but allowed activity in running wheel and given daily handling.
§§ Animals in isolation cages, controls for activity plus handling.

differed from the IC group as much as did the EC group (which lived in the brightly illuminated room). In weight of occipital cortex, the ECI group exceeded the ECT group slightly but not significantly. Thus it appears that the level of ambient illumi-

nation does not play any discernible role. (The EC group in this experiment was peculiar in that its percentage weight difference from IC was smaller in occipital cortex than in total cortex. Three of the 11 EC animals had unusually small weights of occipital cortex, for unknown reasons. AChE/weight of this group, however, is seen in Table VII to follow the usual pattern.)

Effects of IC under high illumination

In another experiment 2 groups of isolated S_1 rats were kept under high ambient illumination, while a 3rd isolated littermate group was kept in the usual dimly lighted IC room; there was also a standard ECT group. The experiment was designed to test possible effects of locomotor activity (in running wheels) and daily handling on animals isolated in individual cages. An Activity-plus-Handling group and a Control group lived in individual cages in the large brightly lighted EC room.

Results. Some of the results are given in Table VIII. In both weight and AChE/weight of the occipital cortex, the ECT group differed significantly from the other 3 groups, but the 3 isolated groups did not differ significantly among each other. Neither activity plus handling nor differences in illumination produced cerebral differences among the isolated groups on these brain measures. Thus, ambient illumination had no perceptible effect among restricted-experience animals, just as in the preceding section it had no measurable effect on enriched-experience animals.

Intermittent illumination without reward

Differences in ambient illumination over a wide range might be expected to have rather little effect, since the animals would be able to adapt to the existing level. Intermittent illumination might be a more effective stimulus, and we have therefore employed it in a number of experiments.

Early in 1962 we added the following new toy to the set of ECT objects: A box 15 cm high, 12.5 cm wide, and 12.5 cm deep, with a flashlight bulb mounted near the top of the front panel; the bulb is illuminated during the time that an animal depresses a sensitive lever in the middle of the front panel. Some visitors to the laboratory, on seeing this toy which has not been described in our previous publications, have asked whether the stimulation it affords might be responsible for the relatively large magnitude of the effects in the occipital cortex. The next paragraph provides evidence on this point.

Results. The 3 ECT–IC experiments run before this light box was introduced yielded a mean percentage difference in weight of occipital cortex of 7.9% ($P < 0.001$). In the 9 experiments in which the light box was used, the average ECT–IC percentage difference in weight of occipital cortex amounted to 5.6% ($P < 0.001$). The difference between these two percentages is not significant. Thus, although the animals do turn on the light intermittently, especially at night (an average of less than 50 times a night per cage), this stimulation did not enhance the ECT–IC effects.

Intermittent illumination with reward

It seemed possible that intermittent illumination might be more important to the animals if it was related to food reward. We therefore ran 3 experiments with operant training where intermittent illumination was employed. In these experiments one animal of each of 9 to 12 litters of the S_1 strain received operant training in a Skinner box, learning to solve a variety of problems, mostly to changing visual stimuli. Its littermate, in a nearby Skinner box with constant dim illumination, received a pellet 'free' whenever the first animal earned one. These conditions were maintained in most cases for 2 h a day, and the animals obtained almost all of their food in the experimental conditions. After a month or more under these conditions, the brains of the littermates under the two conditions were compared.

The results gave meager evidence of effects of the differential experience in the occipital cortex, but somewhat stronger effects in total cortex. No significant effect was found consistently from one experiment to another. In each of the experiments, AChE/weight in occipital cortex was slightly higher in the experimental than in the control animals, whereas the usual EC effect is a lowering of AChE/weight. When analyses of variance were run on the 3 experiments combined, the only significant effect in the occipital region was a 2.1% increase in ChE/weight. In total cortex, there were several significant differences, the experimentals exceeding the controls by 2.6% ($P < 0.05$) in total AChE activity, by 1.8% ($P < 0.05$) in ChE/weight, and by 2.9% ($P < 0.01$) in total ChE activity. It therefore appears that operant training with primarily visual stimuli has relatively little effect on occipital cortex, although there are some significant effects on total cortex.

DISCUSSION

The generality of effects of differential environment on the brain measures reported here and the stability of the pattern of changes across cortical regions deserve comment. We have found similar effects whether experiments lasted 30 or 80 days, whether the experiments were started at 25, 60 or 105 days of age, whether animals had 2 h or 24 h of enriched experience per day. Similar results were also obtained with 3 strains of rats, and with both males and females. This generality becomes more striking when we consider that other cerebral effects of experience are quite specific to strain and sex. Such specificity is found, for example, in changes of brain amines of mice subjected to isolation or grouping; different strains and the two sexes respond quite differently[6].

We asked at the outset whether the particular visual conditions of our experimental situations might be responsible for the fact that larger ECT–IC differences were observed in the occipital cortex than elsewhere in the brain. The experiments considered here indicate that the answer is *No*. Even blinded rats showed a significant ECT–IC difference in weight of the occipital cortex in favor of the ECT group (Table IV).

The effects of environmental enrichment *vs.* impoverishment cannot be reduced

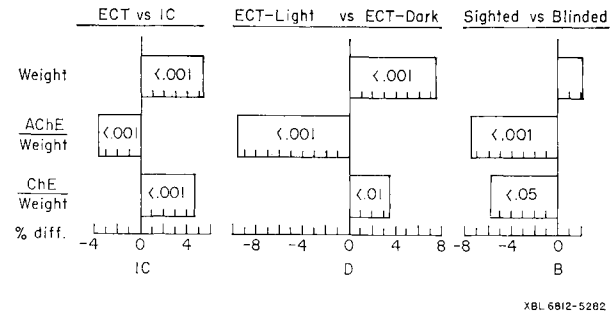

Fig. 1. Effects of 3 sets of treatments on weight and enzymatic activity of occipital cortex of S_1 strain rats. All treatments were maintained from 25 to 105 days of age. The ECT–IC results are based on 98 littermate pairs. Results of comparing ECT in normal light cycle with ECT kept in complete darkness are based on 31 littermate pairs. The Sighted–Blinded comparisons for weight and AChE are based on 20 litters, each litter furnishing 2 sighted rats (S–ECT and S–IC) and 2 blinded rats (B–ECT and B–IC); the Sighted values combine S–ECT and S–IC, and the Blinded values combine B–ECT and B–IC. The ChE Sighted–Blinded value is based on only 9 litters of 4 rats each, that is, 18 sighted and 18 blinded rats. Percentage differences between the first and second treatment of each set are referred to the second member (IC, Dark, Blinded) as baseline. The statistical significance of each difference is shown in the appropriate bar; only the weight difference between Sighted and Blinded was not significant. Differences among the three patterns of effects are discussed in the text.

to either the effects of normal visual experience *vs.* dark-raising nor to effects of sightedness *vs.* blindness, even though the first member of each pair provides richer experience than the second. Differences among the resultant patterns of effects in occipital cortex are shown in Fig. 1. The increases or decreases are taken for the first member of a pair of treatments *versus* the second member; thus the baselines of the 3 parts of the figure are the IC condition, the dark-raised condition, and blindness, respectively. The ECT–IC pattern differs from the Sighted–Blinded pattern on all 3 measures: ECT–IC shows a significant increase in weight of occipital cortex, while S–B does not. The drop in AChE/weight for ECT–IC is smaller in percentage terms than is the gain in weight, while for S–B the drop in AChE/weight is larger than the gain in weight; therefore total AChE (not shown in the figure) is increased in ECT but is significantly decreased by sightedness (*vs.* blindness). ChE/weight (and total ChE activity) are increased by ECT but decreased by sightedness. (The Sighted–Blinded ChE results are the only ones in the figure that are based on just a single experiment, so, although they are statistically significant, they are not as solidly established through replication as are the other results in the figure.) The results of light-cycle *vs.* dark-raising are like those of ECT–IC in both tissue weight and ChE activity, but they resemble those of S–B in the relatively large drop in AChE activity. It is therefore clear that each of these three ways of altering experience produces its own pattern of cerebral effects and that one cannot be reduced to another.

The results of our experiments leave us with the problem of determining what aspects of stimulation and experience other than visual may account for the EC–IC effects and of trying to find out why the occipital cortex is the region in the rat brain that is most susceptible to showing such changes. Concerning the effective stimulation,

there remain two possibilities: (a) A particular sensory modality other than vision (*e.g.*, olfaction) may be responsible, or mainly responsible, for inducing the EC–IC differences in blinded or dark-raised rats. (b) Any of several modalities, or a combination of any of several, may be adequate to induce the cerebral effects. Just as it had been demonstrated many years ago that a rat can solve a maze using any of several modalities and can do better when a number are available[7], so there may be multiple channels serving the EC–IC effects. In either case, it will be desirable to study peculiarities of the occipital cortex of the rat to determine what renders this area more responsive than other regions to sensory or experiential input.

SUMMARY

1. When rats were exposed to enriched or impoverished environments, the enriched-experience animals developed significantly greater cortical measures than their restricted littermates: tissue weight (Table I), total AChE activity (Table II), total ChE, and cortical depth (Table III). Most of these differences were significantly greater in the occipital region of the cortex than in other cortical areas (see Table I, II and III). The larger size of the occipital effects occurred as clearly in adults as in young animals, in both 30-day and 80-day experiments, in exposure to the enriched condition for 2 h a day as well as for 24 h a day, in all the strains of rats tested and in both sexes.

2. Visual experience is not a necessary component of the conditions that induce changes in occipital and other cortical regions. Significant brain differences arise between littermates kept in enriched or impoverished environments even if the animals are blinded (Table IV) or are kept in total darkness (Table VI).

3. While significant changes are induced in occipital cortex by blinding (S *vs.* B) and by dark-raising (L *vs.* D), these two patterns of effects differ from each other, and both differ from the changes induced by variation of environmental complexity (EC *vs.* IC) (see Fig. 1).

4. Intermittent visual stimulation related to operant reinforcement was not an effective condition for producing changes in the occipital cortex, whereas a larger number of significant effects of training was found in total cortex.

5. Other species of rodents — mice and gerbils — also show significant brain differences as a result of enriched or impoverished experience, but the occipital effect does not appear to predominate as clearly in these species as it does in the rat. Thus the problem of the special plasticity of the occipital area should be investigated specifically in the rat.

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