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Effects of Successive Environments on Brain Measures[1]

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BENNETT, E. L., M. R. ROSENZWEIG, M. C. DIAMOND, H. MORIMOTO AND M. HEBERT. *Effects of successive environments on brain measures.* PHYSIOL. BEHAV. 12(4) 621–631, 1974. – To test the persistence of cerebral effects induced by differential experience, littermate rats were placed in an enriched environment (EC) or an impoverished environment (IC) for either 30 or 80 days. Following this initial period, one rat of each litter was transferred from the EC to IC condition while its littermates remained in EC or IC. The second phase lasted either 7, 14, 21, 32 or 47 days. The cerebral differences induced by differential experience began to dissipate when the animals were placed in a common environment, but statistically significant differences still persisted weeks after the end of the inducing conditions. Greater persistence was found after 80 days of initial exposure than after 30 days. Following 80 days in EC, significant persistence of brain weight differences was found 21 days after removal from EC to IC; significant persistence of differences in acetylcholinesterase and cholinesterase activities were found 47 days later. Different brain regions and different measures showed somewhat different patterns of results. Three different kinds of environmental enrichment – devised in three laboratories – were compared for their effects on brain weights and brain enzymes.

Enriched environment Impoverished environment Environmental enrichment Persistence of brain effects Rat Brain weights Brain enzymes Acetylcholinesterase Cholinesterase

IN RECENT years a number of investigators have found that exposing rodents to enriched or impoverished environments for periods ranging from days to months leads to significant changes in brain chemistry, brain weights, and brain anatomy (for review see [22]; also [9, 11, 14, 15, 25, 26, 27]. The present paper is concerned with the persistence of changes produced by enriched experience after animals are placed in an impoverished condition. Do the cerebral changes induced by this differential experience disappear rapidly when the animals are removed from the experimental environment, or are the changes essentially fixed and permanent? Our findings do not support either of these extreme positions.

Since we find rather prolonged persistence of effects whereas Brown [3] did not, Experiment 2 will compare the relative effectiveness of Brown's enriched condition and the Berkeley EC condition in producing changes in brain weights and brain enzymes. A third enriched condition, that designed by Ferchmin *et al.* [9], will also be included in this comparison. Subsequent papers will deal with investigations of (a) how soon cerebral changes can be detected after exposure to differential environments, and (b) the effects of training and testing procedures over several days in a learning situation on relative brain measures of animals previously maintained in enriched or impoverished environments.

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EXPERIMENT 1

EFFECTS OF TRANSFERRING RATS FROM ENRICHED TO IMPOVERISHED ENVIRONMENT

Only four studies appear to have investigated the effects on brain measures of transferring rats from an enriched to an impoverished environment [3, 10, 21, 28], and this topic has not yet been treated adequately. Zolman and Morimoto [28] sacrificed two groups of rats, some directly after 30 days in the enriched (EC) or impoverished conditions (IC), others after removal from EC to IC for 30 days before sacrifice. Both acetylcholinesterase (AChE) activity and tissue weight of samples of the cerebral cortex and the rest of the brain were measured. Although the immediate sacrifice groups showed larger differences between EC and IC animals than did the delayed sacrifice groups, the latter, nevertheless, still yielded statistically significant differences in subcortical AChE activity and in the brain weight measures. Thus, some of the EC vs. IC effects appeared to persist for at least 30 days. Rosenzweig *et al.* [21] reported briefly on an experiment in which animals were placed in either EC or a condition of isolation in extreme impoverishment (IEI) for 80 days, after which some rats remained in the same condition for another 50 days while other rats were transferred to the opposite condition. The differences in cortical weight induced by the initial 80-day period did not show significant persistence at the end of the following 50-day reversal period.

Geller [10] maintained rats in EC and IC conditions for 30 days and then rehoused the animals in pairs for an additional 30 days. Analyses were made of a number of neurotransmitters and enzymes involved in their metabolism, as well as brain and body weight measures. No significant difference was found in total brain weight. Although significant differences were still found in dopamine and norepinephrine concentrations, they were smaller than the differences after the 30-day exposure to differential environments. It was concluded that the effects of the environment are reversible and tend to disappear.

Brown [3] measured AChE and cholinesterase (ChE) activities in the occipital cortex after rats had spent 80 days in a visually enriched condition (intensive visual stimulation – IVS) or in the standard colony condition (SC). She reported very large differences between IVS and SC when the rats were sacrificed directly from the differential environments, but replacing IVS rats into SC led to rapid diminution of the effects. After 3 days there was no longer a significant effect in AChE (although the percentage difference at this time was still large – 18%), and after 7 days there was no longer a difference in ChE.

We decided to study the persistence of cerebral effects more fully because of the inconsistent results of previous studies. Furthermore, each of the previous studies employed only a single length of initial differential experience and we wanted to test whether persistence varied with the length of differential experience. Finally, we had some reservations about the paper of Brown, both because of the low enzymatic values reported and because of the surprisingly large differences in enzymatic activities between groups and the large variabilities within groups.

METHOD

Experimental Design

Each experiment consisted of an initial phase of differential experience in the enriched (EC) or impoverished (IC) conditions, followed by the second phase in which some rats were transferred from EC to IC. In each experiment there were three groups of littermates: (a) Animals kept in EC in both phases (designated as EC·EC), (b) animals in EC during the first phase and then in IC during the second phase (EC·IC), and (c) animals in IC during both phases (IC·IC). (A full design would have also included an IC·EC group, but our main concern was with the persistence of differences induced with respect to the IC baseline.) The first phase lasted either 30 or 80 days; the second phase was varied from 7 to 47 days. Differences between groups were expressed as

$$100 \times \frac{\text{EC·EC} - \text{IC·IC}}{\text{IC·IC}} \quad \text{and} \quad 100 \times \frac{\text{EC·IC} - \text{IC·IC}}{\text{IC·IC}}$$

while persistence of effect after return to IC was measured by the ratio of differences in cerebral measures between EC·IC and IC·IC to those between EC·EC and IC·IC; that is,

$$\% \text{ persistence} = 100 \times \frac{(\text{EC·IC} - \text{IC·IC})}{(\text{EC·EC} - \text{IC·IC})}$$

Environmental Conditions

EC animals were housed in a group of 12 in a large cage (70 × 70 × 46 cm). The cage was provided with stimulus objects (toys) from a standard pool. Each day there were six or more toys in the EC cage; some were in new positions from the previous day and some were newly taken from the pool. (Rats in an EC cage are shown in Fig. 1 of [1]; the pool of objects is shown in Fig. 1 of [19].) Each day the EC animals were placed for 30 min in the field of a Hebb-Williams apparatus (75 × 75 cm) where the pattern of barriers was changed daily.

IC animals were housed in individual cages (32 × 20 × 20 cm) with solid sidewalls that provided visual isolation. The IC cages were placed in a separate, quiet, and dimly illuminated room. In these special IC cages, the floors were made of wire bars; pans of shavings below the cages were changed without touching the animals.

Both EC and IC animals had laboratory pellets and water available ad lib. All animals were weighed regularly, usually at weekly intervals.

In each experiment littermate male triplets of the Berkeley S_1 strain were assigned at weaning (about 25 days of age), semirandomly to three groups. Assignment was semirandom to insure that the distributions of body weights among groups would be similar. Animals whose weights departed more than 15% from the littermate were excluded. The groups were then assigned at random to the experimental conditions.

Removal and Weighing of Brain Tissue

At the end of the experiment, the animals were put in a multiple-unit cart bearing code numbers that did not reveal the experimental condition of any rat. The animal was decapitated, and the brain was dissected following our standard procedures [24]. Using a calibrated plastic T-square, we removed standard samples of occipital and somesthetic cortex. The other brain sections were the following: remaining dorsal cortex; ventral cortex, including the hippocampus and corpus callosum; cerebellum and medulla;

remaining subcortical brain, including the olfactory bulbs. Measures from all of the cortical sections were combined to give total cortex; measures from the cerebellum and medulla and remaining subcortical brain area combined to give rest of brain.

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

Chemical Analysis

The quantitative method of Ellman, Courtney, Andres, and Featherstone [8] has been adapted for the differential assay of acetylcholinesterase (AChE) and cholinesterase (ChE). Our procedure has been described in more detail in Rosenzweig and Bennett [20]; a complete description can be obtained from the authors upon request.

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

Statistical Tests

Results of individual experiments were evaluated by two-way analyses of variance (litters vs. treatments). Overall results combining several experiments utilized the same design with replication. Comparisons between different experimental groups were done by Duncan's multiple-range test.

RESULTS

Brain Weight Effects

Since most of the results of the 11 experiments will have to be presented in brief summary form, it will be helpful at the outset to inspect detailed results for two representative experiments. The results are given for the two experiments in which the initial EC or IC experience lasted for 80 days, after which one EC group was transferred to IC for 21 days. Table 1 presents percentage differences between EC·EC and IC·IC and between EC·IC and IC·IC groups.

The EC·EC versus IC·IC comparison is like the EC versus IC comparison in many experiments that we have run (summarized in Table 7 of [22]), except that we have not previously used a 101-day period. As in our previously reported EC-IC experiments, the present EC·EC groups exceed their littermate IC·IC groups significantly in weight of the cortical samples and in the weight of cortex to rest of brain. The ratio of cortex to rest of brain weight has proved to be one of the most stable measures of EC-IC differences.

In the second experiment in Table 1, the differences between EC·EC and IC·IC were relatively large and highly significant; the EC·IC versus IC·IC differences were about half as large, and several of them were also statistically significant. In the first experiment, for unknown reasons, the EC·EC versus IC·IC effects were smaller than in the second, and the EC·IC versus IC·IC effects were again about half of the foregoing magnitudes and most were not significant. When results for the two experiments were combined, EC·IC was found to differ significantly from IC·IC on weights of each of the cortical sections except ventral cortex, and there was a highly significant difference on the cortex/rest of brain ratio. These differences between EC·IC and IC·IC occurred despite the fact that the two groups had both been in the IC environment for the 21 days before sacrifice. The overall persistence measures show that about 50% of the cortical weight differences induced by the initial 80-day period were still present after 21 succeeding days in IC. (Because the rest of brain has not shown regular effects in a large series of experiments, we have not given percentage persistence values for this measure.)

TABLE 1

PERSISTENCE OF BRAIN WEIGHT EFFECTS FOR 21 DAYS AFTER AN INITIAL 80-DAY PERIOD OF DIFFERENTIAL EXPERIENCE (PERCENTAGE DIFFERENCES BETWEEN GROUPS)

Sacrifice Date	N (litters)		Cortex					Rest of Brain	Total Brain	Cortex /Rest
			Occipital	Somesthetic	Rem. Dorsal	Ventral	Total			
Expt. 1	11	EC·EC vs IC·IC	11.4†	0.0	7.0†	4.2	5.8†	−0.7	2.0	6.4‡
7/10/72		EC·IC vs IC·IC	4.6	6.1*	3.2	1.6	3.0	−1.0	0.6	3.9†
		% Persistence	40	–	46	38	52	–	30	61
Expt. 2	13	EC·EC vs IC·IC	15.8‡	5.2*	7.4‡	7.1‡	8.0‡	0.0	3.3*	7.9‡
9/18/72		EC·IC vs IC·IC	7.5†	1.7	3.5	3.9*	3.9†	−0.3	1.4	4.2‡
		% Persistence	47	33	47	55	49	–	42	53
Combined	24	EC·EC vs IC·IC	13.8‡	2.9	7.2‡	5.8†	7.0‡	−0.3	2.7*	7.2‡
		EC·IC vs IC·IC	6.2†	3.7*	3.4*	2.8	3.5†	−0.7	1.1	4.1‡
Combined		% Persistence	45	128	47	48	50	–	41	57

*$p<0.05$ †$p<0.01$ ‡$p<0.001$

TABLE 2

PERCENTAGE DIFFERENCES AMONG GROUPS IN BRAIN WEIGHT AS A FUNCTION OF DURATION OF INITIAL AND SECOND PHASES

Persistence Period	Sacrifice Date	N (litters)	% Difference EC·EC vs IC·IC			% Difference EC·IC vs IC·IC		
			Occip. Cortex	Total Cortex	Cortex /Rest	Occip. Cortex	Total Cortex	Cortex /Rest
A. After 30-Day Initial Phase								
7 days	5/12/71	12	6.4†	2.8*	4.8‡	4.3*	1.3	2.7†
	10/20/71	12	5.7*	6.4‡	2.6*	7.7†	3.0*	0.8
	Combined	24	6.1‡	4.6‡	3.6‡	6.0‡	2.1*	1.8†
14 days	2/2/71	12	12.0‡	7.6‡	3.2†	2.2	1.0	1.4
	3/31/71[a]	11	4.1	5.1‡	4.1‡	0.4	2.5*	2.2*
		11				1.2	1.5	0.8
	Combined	23/34	8.0‡	6.4‡	3.6‡	1.2	1.7*	1.5*
B. After 80-Day Initial Phase								
7 days	12/9/71	12	7.2*	2.2	7.0‡	5.0	3.4†	4.4‡
14 days	12/16/71	10	1.0	6.0‡	2.9*	0.8	4.8†	2.1
	6/26/72	12	9.1*	6.4‡	5.4†	1.8	2.6*	1.4
	Combined	22	5.4*	6.2‡	4.2‡	1.3	3.6‡	1.7
21 days	7/10/72	11	11.4†	5.8†	6.4‡	4.6	3.0	3.9†
	9/18/72	13	15.8‡	8.0‡	7.9‡	7.5†	3.9†	4.2‡
	Combined	24	13.8‡	7.0‡	7.2‡	6.2†	3.5†	4.1‡
32 days	11/27/72	12	7.7*	3.5†	3.8‡	3.0	0.6	1.5
47 days	2/26/73	12	6.2*	4.4*	4.9‡	1.6	1.5	2.0

[a]This experiment had two EC·IC groups *$p<0.05$ †$p<0.01$ ‡$p<0.001$

Now let us see how the effects vary as a function of the duration of the second phase (7, 14, 21, 32 or 47 days). The main results are given in Table 2, and percent persistence values are shown graphically for occipital cortex and cortex/rest of brain in Fig. 1. When the initial EC experience lasted only 30 days, persistence was rather short-lived, as will be seen in the upper section of the table. There were significant effects 7 days after the switch from EC to IC. The second 14-day experiment included two EC·IC groups. The combined 14-day values show that by this time the effects of 30 days of differential experience had dropped considerably below the 7-day percent persistence values; nevertheless, EC·IC still differed significantly from IC·IC in weight of total cortex and in the weight ratio of cortex to the rest of the brain.

The effects of 30 days of differential experience appeared to be less persistent than those of 80 days (Table 2 and Fig. 1). The curves represent our best estimates of the trends of the data. It is interesting to note that the 34% persistence (1.5%/4.4%) for total cortex weight after 47 days is very close to the 30% reported after 50 days in our 1967 paper [21].

Effects on Chemical Measures

In EC vs. IC experiments we have typically found AChE/weight values to be significantly lower in the cortex of EC rats than in that of IC littermates, and this negative effect is usually largest in the occipital area; non-cortical brain regions show little or no consistent EC-IC difference in AChE/weight. This typical pattern was found in the present EC·EC versus IC·IC comparison, and a similar pattern with smaller but still signficant effects is found in the EC·IC versus IC·IC comparisons. The less specific

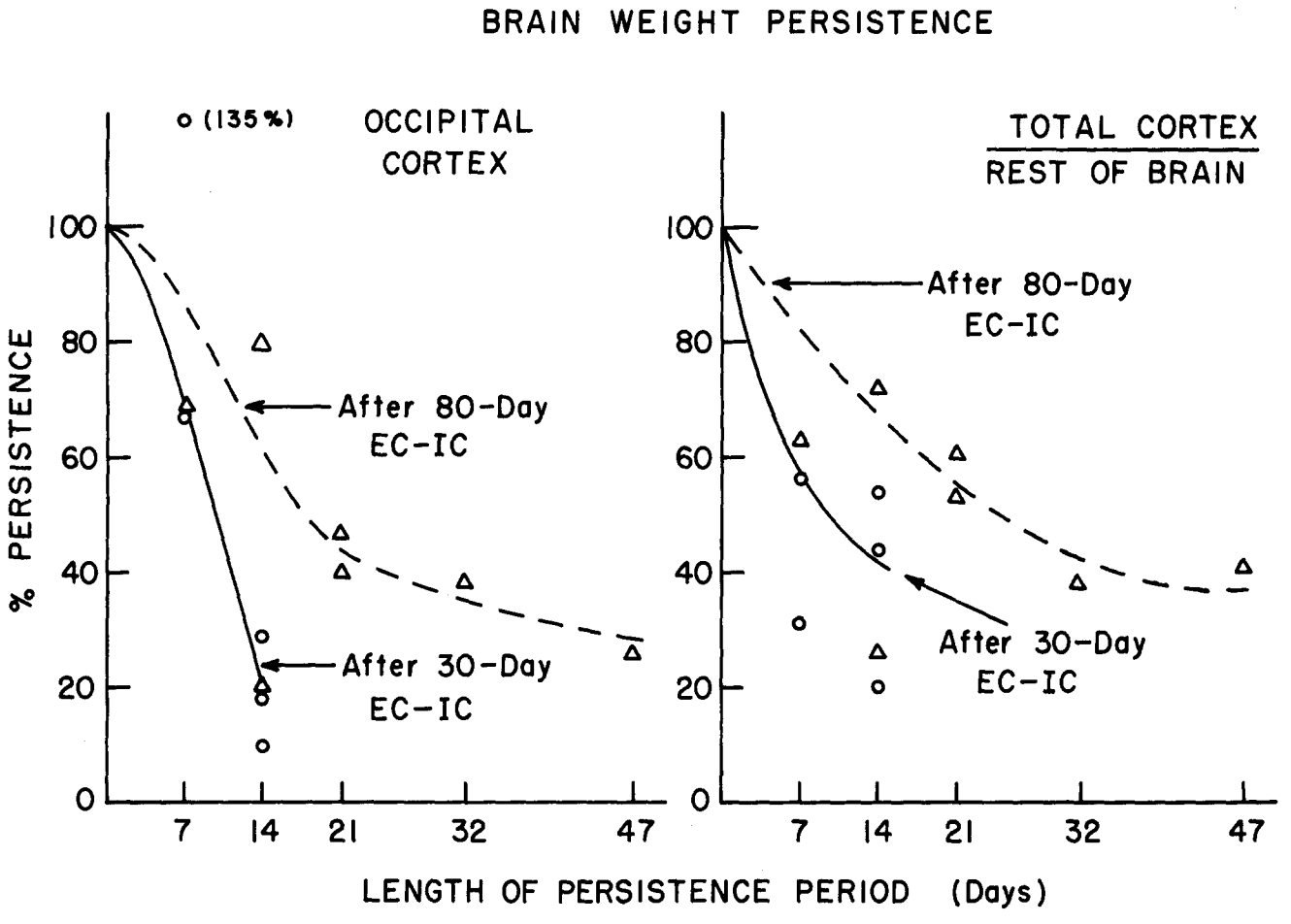

FIG. 1. Persistence of brain weight for occipital cortex and for the ratio Total Cortex/Rest of Brain of rats transferred to IC after 30 days in EC o ——— o and after 80 days in EC △ - - - - △.

enzyme ChE typically yields a different pattern of results. ChE/weight is usually greater in EC than in IC throughout the brain, although the differences are not as consistent as those of AChE. The lack of consistency of ChE effects was again found in the present case where the EC·EC vs. IC·IC differences were not significant. (Interestingly, two of the EC·IC vs. IC·IC differences were significant; it is shown below that differences in ChE activity often increased during the persistence period.) Finally, the ratio of ChE to AChE activities gives a purely chemical measure in which tissue weight is cancelled out. This measure is usually greater in EC than in IC throughout the brain, and especially in the cerebral cortex and in the cortex/rest of brain ratio. In the present experiments the differences in ChE/AChE values are highly significant. With regard to persistence of the chemical effects, the results of Table 3 show that when animals spent 80 days in EC and then 21 days in IC, many of the enzymatic brain values still differed significantly from IC values; this finding was especially true for the occipital cortex.

Now let us see how persistence in AChE effects varies as a function of length of the initial and second phases of the experiment (Table 3 and Fig. 2). Following the 30-day initial phase, signficant persistence effects were found after 7 days in occipital cortex, total cortex and the cortex/rest of brain ratio. But after 14 days the persistence had dwindled, and only in the cortex/rest of brain ratio was EC·IC still significantly different from IC·IC. In contrast, there was considerably greater persistence after the 80-day initial period (Fig. 2). In the occipital cortex, the EC·IC value differed from IC·IC at beyond the 0.05 level for all four persistence periods for which chemical analyses were made, and it never fell below 65%, whereas Brown [3] reported a loss of significance in AChE activity after only 3 days. In the cortex/rest of brain ratio of AChE, significance was seen after 7, 14, and 21 days, but only at the 0.10 level at 47 days.

The results for ChE activity per unit of weight are more variable from experiment to experiment than for the other measures, and these experiments yielded few significant EC·EC versus IC·IC effects, even for the 80-day period. The ChE results will therefore not be presented in detail, but some will be noted here, since the pattern of persistence for ChE differed from those described above for tissue weight and for AChE/weight. Following 80 days of EC or IC, significant positive EC·IC versus IC·IC differences were found in ChE activity of occipital cortex after persistence periods of 14 days, 21 days, and 47 days. For both the 21-day and 47-day persistence periods, the percentage persistence values were over 100 (21 days, 344%,

TABLE 3

PERCENTAGE DIFFERENCES AMONG GROUPS IN ACETYLCHOLINESTERASE ACTIVITY AS A FUNCTION OF DURATION OF INITIAL AND SECOND PHASES

Persistence Period	Sacrifice Date	N (litters)	% Difference EC·EC vs IC·IC Occip. Cortex	% Difference EC·EC vs IC·IC Total Cortex	% Difference EC·EC vs IC·IC Cortex /Rest	% Difference EC·IC vs IC·IC Occip. Cortex	% Difference EC·IC vs IC·IC Total Cortex	% Difference EC·IC vs IC·IC Cortex /Rest
A. After 30-Day Initial Phase								
7 days	5/12/71	12	−2.4	−1.9	−4.5†	−3.8*	−1.7	−3.7†
	10/20/71	12	−1.7	−2.6	−2.2	−1.7	−2.0	−2.2
	Combined	24	−2.1	−2.2*	−3.3†	−2.8*	−1.8*	−2.9†
14 days	2/2/71	12	−6.0†	−4.3†	−3.4†	−1.2	−1.1	−2.6*
	3/31/71	11	−5.4‡	−3.6*	−4.5‡	−2.2	−1.9	−2.7†
	Combined	23	−5.7‡	−4.0‡	−3.9‡	−1.7	−1.5	−2.6‡
B. After 80-Day Initial Phase								
7 days	12/9/71	12	−4.8‡	−1.6	−5.2‡	−3.6†	−2.2	−3.2*
14 days	12/16/71	10	−6.7‡	−4.0†	−4.5†	−5.4†	−3.2*	−3.0*
21 days	7/10/72	11	−6.1†	−3.7	−5.1†	−5.4†	−2.3	−2.5
	9/18/82	13	−6.9‡	−6.4‡	−6.6‡	−4.5†	−2.7*	−2.3*
	Combined	24	−6.5‡	−5.2‡	−5.9‡	−4.9‡	−2.5*	−2.4†
47 days	2/26/73	12	−4.5*	−4.7‡	−6.4‡	−4.2*	−2.3	−2.1

*$p<0.05$ †$p<0.01$ ‡$p<0.001$

$p<0.01$; 47 days, 167%, $p<0.01$). It appears that differences in ChE activity induced by EC and IC experience were not only more persistent than differences in brain weights or in AChE activity but that EC-IC differences in ChE activity often actually increased during the persistence period.

Results for the purely chemical ChE/AChE ratio are given in Table 4. The EC-IC difference on this ratio is usually positive, and such results are seen for all of these experiments except 7-days-after-30 days. The two 14-day-after-30-day experiments yielded typical measures and showed significant persistence effects. At all four durations after the initial 80-day period, both occipital cortex and total cortex showed significant EC·IC versus IC·IC differences. The cortex/rest of brain ratio yielded significant differences at 7 and 14 days, following 80 days.

EXPERIMENT 2

CEREBRAL EFFECTS OF THREE KINDS OF ENRICHED EXPERIENCE

The foregoing results demonstrate significant persistence of cerebral effects of differential experience, even for weeks after rats are returned from the EC to the IC environment. Since these results were not in conformity with the rapid disappearance of effects that Brown [3] reported, we wondered whether the cerebral effects obtained in our experimental environments could properly be compared with effects of her rather different environmental conditions. We also noted that Brown claimed very large differences in enzymatic activity in occipital cortex after 80 days in her enriched and colony condition, -34% in AChE/weight and 144% in ChE/weight. Our search for conditions that would yield large differential effects provided another reason to compare directly the cerebral effects of Brown's "intensive visual stimulation" (IVS) and our enriched condition (EC) within the same experiment. A third enriched condition, that of Ferchmin *et al.* [9], was also included, since it affords a greater variety of stimulation than either the Brown or the Berkeley conditions.

METHOD

Animals

Forty-eight male rats of the inbred Fischer line were matched by weight into groups of four. From each group,

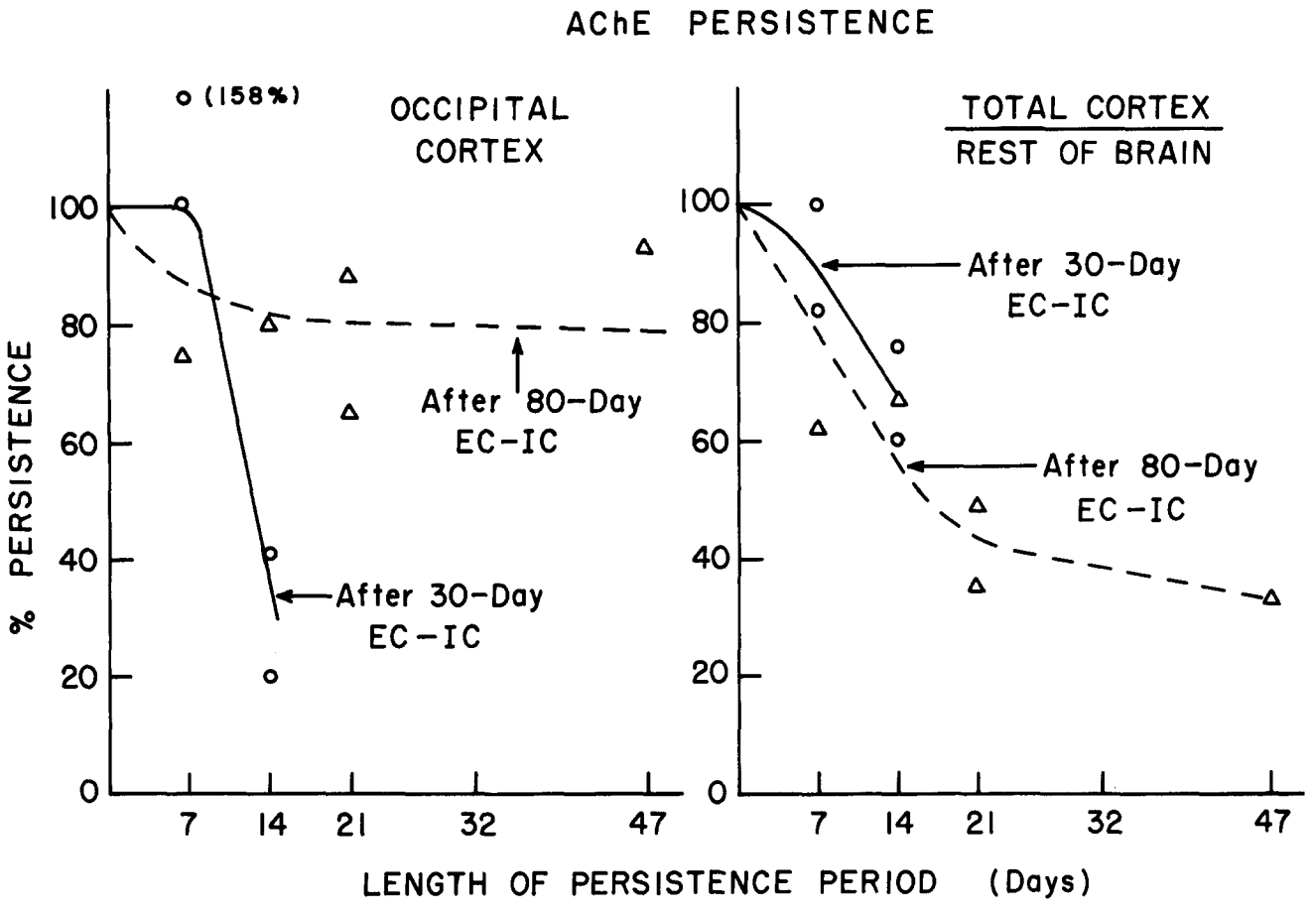

FIG. 2. Persistence of AChE activity for occipital cortex and for the ratio Total Cortex/Rest of Brain of rats transferred to IC after 30 days in EC o ——— o and after 80 days in EC △ - - - - △.

one rat was assigned randomly to each of the four experimental conditions. The animals were assigned at about 30 days of age and were kept in the differential environments until sacrificed at about 60 days of age.

Environmental Conditions

The four conditions were these: (a) The standard Berkeley Impoverished Condition (IC). (b) The standard Berkeley Enriched Condition (EC). (c) Intensive Visual Stimulation (IVS); this resembled closely the condition used by Brown [3]. In brief, the stimulus objects were irregularly shaped flat plastic and metal forms shown in Fig. 1 of Brown. (Personal communication revealed that Brown's objects were 10 times the size shown in her figure rather than the 6 times indicated on the scale, because the scale had been reduced in reproduction. Our objects correspond to Brown's actual objects.) Twelve different objects out of a pool of 36 were hung in the cage each day. Our procedures differed somewhat from those of Brown in that we housed 12 rats in the cage instead of 10 and that we used our standard EC cage which is somewhat larger than the cage used by Brown. Also, since we have found that four weeks produces most of the changes observed with much longer periods, our experiment lasted 28 days instead of the 80 days of Brown's experiment. The Fischer rats we used are albinos, as are the Sprague-Dawley rats used by Brown. (d) Ferchmin Enriched Condition (FEC) modeled after the condition described briefly by Ferchmin, Eterovic and Caputto [9] and described more fully in personal communication with the authors. Six different cages were used for FEC. Two cages were small – the two halves of a Berkeley EC cage divided down the middle by an opaque partition. The third cage was a normal EC cage, and the fourth was an EC cage placed on its side. After the first two weeks of the experiment, a fifth and sixth cage were added – a tall cage (170 x 40 x 40 cm) and a broad cage (105 x 123 x 41 cm). In order to facilitate the exploration of the FEC rats early in the experiment, they were given guides from the second through seventh days. The guides were young S_1 rats who already had a week of experience with the EC cages and toys. The animals were divided between two cages at any given time, and at each change of cages the animals were regrouped so that each rat met every other rat and each rat experienced all the cages and a variety of toys. The Fischer and S_1 rats got along well, often aggregating together. On the initial day of the experiment, the 12 FEC rats were placed in the divided EC cage, six in each half of the cage; each half contained a small metal house and 8 other toys. On the morning of the second day, 6 FEC rats and 4 guides were placed in each half of the cage. That afternoon, 6 FEC rats and 4 guides were placed in each of

TABLE 4

PERCENTAGE DIFFERENCES AMONG GROUPS IN ChE/AChE RATIO AND PERSISTENCE VALUES AS A FUNCTION OF DURATION OF INITIAL AND SECOND PHASES

Persistence Period	Sacrifice Date	N (litters)	% Difference EC·EC vs IC·IC			% Difference EC·IC vs IC·IC			% Persistence		
			Occip. Cortex	Total Cortex	Cortex /Rest	Occip. Cortex	Total Cortex	Cortex /Rest	Occip. Cortex	Total Cortex	Cortex /Rest
A. After 30-Day Initial Phase											
7 days	5/12/71	12	0.3	3.0	2.8	0.3	0.4	1.2	100	13	43
	10/20/71	12	−1.2	1.5	1.5	−2.0	1.2	0.9	167	80	60
	Combined	24	−0.6	2.2	2.1	−1.0	0.8	1.1	167	36	52
14 days	2/2/71	12	6.6*	4.3†	3.7*	3.3	2.2	2.5	50	51	68
	3/31/71	11	8.4†	4.3*	4.2†	4.2	3.3	2.6	50	77	62
	Combined	23	7.5‡	4.3‡	3.9‡	3.7*	2.8*	2.6*	49	65	67
B. After 80-Day Initial Phase											
7 days	12/9/71	12	6.6†	4.5*	5.1†	4.2*	4.1*	3.7*	64	91	72
14 days	12/16/71	10	3.2	2.2	4.6†	9.9‡	4.9*	4.6†	309	223	100
21 days	7/10/72	11	4.6	3.2	3.9	8.0†	3.0	1.6	174	94	41
	9/18/72	13	11.1‡	8.4‡	6.4‡	9.0‡	4.6†	2.5	81	55	39
	Combined	24	8.1‡	6.1‡	5.3‡	8.6‡	3.9†	2.1	106	64	40
47 days	2/26/73	12	7.8‡	6.4‡	7.3‡	9.4‡	3.9*	2.6	120	61	36

*$p<0.05$ †$p<0.01$ ‡$p<0.001$

the two large cages. Thereafter the animals were moved from cage to cage about 8 a.m. and 2 p.m. every day; the pattern of rotation was two successive periods in a large cage followed by one period in a small cage. The Ferchmin procedures were intended to afford a greater variety of stimulation than the standard EC, coupled with a gradual start and encouragement to explore.

RESULTS

Cerebral Effects

The mean values of the IC group and the percentage differences of each of the other groups from the IC means are given in Table 5. When Fischer rats are assigned to enriched or impoverished conditions at weaning, the impoverished-experience group typically gains body weight much more rapidly than the others; the difference in body weights is about twice as large as in the S_1 line. Each enriched group was about 20% less in terminal body weight than the IC group (Table 5). Because brain weights depend in part on body weights, the positive differences in cortical weight between enriched-experience and impoverished-experience rats were rather small in these experiments. Low body weight also reduced subcortical weight, however, so the cortex/rest of brain weight ratios were highly significant, as usual. The largest cortex/rest of brain weight ratio effect was found to result from the Ferchmin EC treatment. This ratio effect was significantly greater than that of our standard EC treatment (FEC vs. EC, $p<0.05$) and marginally greater than the Brown treatment (FEC vs. IVS, $p<0.10$). The EC and IVS treatments yielded almost identical results on the cortex/rest of brain ratio, and there were no significant differences between these two groups on any of the weight measures.

The usual effect of EC versus IC on AChE activity per unit of weight is a decline in the cortex and an increase in the rest of the brain. In the current experiment, the cortical changes were smallest in the EC group and largest in FEC. FEC differed significantly from EC in occipital cortex, somesthetic cortex, remaining dorsal cortex, and total brain. IVS was intermediate between EC and FEC in AChE at most brain regions and did not differ significantly from either on any measure.

The typical increase of ChE/weight in EC versus IC showed up here more clearly than is usual in experiments of four-week length. IVS yielded ChE results very similar to

TABLE 5

COMPARISONS OF EFFECTS OF THREE TYPES OF ENRICHED EXPERIENCE ON BRAIN VALUES (PERCENT DIFFERENCES FROM IMPOVERISHED-EXPERIENCE LITTERMATES)

	Tissue Weight				AChE/Weight			
		% Difference from IC				% Difference from IC		
	IC Mean[a]	EC	FEC	IVS	IC Mean[b]	EC	FEC	IVS
Cortex								
Occipital	74.3	2.8	8.9†	4.9	6.84	−2.8	−6.0‡	−5.3†
Somesthetic	55.7	− 1.2	2.6	1.0	8.10	0.2	−3.6†	−1.7
Rem. Dorsal	325.8	2.3	5.0*	2.6	8.19	0.6	−2.9*	−1.1
Ventral	296.4	− 0.5	3.8	0.4	10.78	1.3	−0.2	0.9
Total	752.2	1.0	4.7*	1.8	9.07	0.5	−2.0	−0.8
Rest of Brain	957.2	− 3.7*	− 2.6	− 3.5*	20.01	4.9‡	3.6†	3.4†
Total Brain	1709.4	− 1.6	0.6	− 1.1	15.20	2.8†	0.8	1.3
Cortex/Rest	0.789	4.9‡	7.5‡	5.5‡	0.453	−4.2‡	−5.4‡	−4.0‡
Body Wt. (gm)	188.3	−21.6‡	−22.7‡	−18.6‡				

	ChE/Weight				ChE/AChE			
		% Difference from IC			IC Mean	% Difference from IC		
	IC Mean[c]	EC	FEC	IVS	× 100	EC	FEC	IVS
Cortex								
Occipital	0.37	6.9‡	0.9	4.7*	5.41	9.9‡	7.3†	10.4‡
Somesthetic	0.40	4.1*	1.3	4.7*	4.92	3.8*	5.1†	6.4‡
Rem. Dorsal	0.40	4.0*	4.0*	4.2*	4.90	3.3*	7.0‡	5.3‡
Ventral	0.38	3.5†	2.0	4.9‡	3.53	2.2	2.2	4.0*
Total	0.39	4.1†	2.7*	4.6‡	4.29	3.6†	4.9‡	5.4‡
Rest of Brain	0.58	2.2	1.8	4.0*	2.92	−2.6	−1.7	0.5
Total Brain	0.50	2.4	1.5	3.6*	3.28	−0.4	0.7	2.3*
Cortex/Rest	0.667	2.2	0.8	0.6	1.47	6.5‡	6.6‡	4.8†

[a]Brain weights in milligrams; body weight in grams.
[b]AChE activity is expressed in terms of nanomoles acetylthiocholine hydrolyzed/minute/milligram.
[c]ChE activity is expressed in terms of nanomoles butyrylthiocholine hydrolyzed/minute/milligram.
*$p<0.05$, †$p<0.01$, ‡$p<0.001$

those of EC and not the very large effects that Brown reported. FEC showed rather small differences from IC in ChE activity; in the occipital cortex the FEC effect was significantly smaller than that of IVS ($p<0.05$).

On the weight-free measure of ChE/AChE, all three types of enrichment brought about highly significant differences from IC. Furthermore, there was little difference among the three enrichment treatments on this measure.

Overall, it appears that all three enrichment treatments are effective, and none of them is uniformly superior to the others. For purposes of comparison, the cortex/rest of brain ratio may be the best measure since it has yielded the most stable and reliable differences among groups in many experiments. On this measure, the FEC treatment is seen to have yielded the largest effects in weight, AChE/weight, and ChE/AChE.

Behavioral Observations

When the enriched-experience groups were replaced in their cages after the daily change of stimulus objects, all of the groups showed a brief period of heightened activity as they inspected the new objects. The designation, "Intensive Visual Stimulation," for the situation of Brown appeared to be a misnomer if it was meant to imply that the objects provided solely or chiefly visual stimulation. The rats were seen to sniff at and bite these objects. Furthermore, the insulation on the wires suspending these objects was frequently found to be chewed, usually a little above the top of the object. We did not observe the rats do this, so it is likely that the insulation was chewed at night; presumably the rats climbed up on the objects in the dark when no visual stimulation was possible. We have shown elsewhere that the usual pattern of EC-IC differences developed when rats were maintained in EC in complete darkness or after having been blinded [23]. It is probable that input through a number of sensory modalities usually contributes to the EC-IC effects; the main modalities probably include touch, smell, audition and vision.

DISCUSSION

The central results of this study are two: (a) The cerebral differences induced by a period of differential experience begin to dissipate when the animals are placed in a common environment, but (b) statistically significant differences still persist weeks after the end of the inducing conditions.

Within these results, there is a good deal of specificity of effects, depending upon both the brain region considered and the brain measure used. That is, the degree of persistence varies not only with the length of the initial and second experimental periods but also with the brain region and brain measure. No one measure provides the full story on persistence; it cannot be concluded that the failure to find a continuing effect on one measure means that the brain has returned completely to its former state and shows no residuum of the previous experience. Even when differences in relatively gross brain measures have dissipated, it is possible that differences still exist in fine anatomical consequences of EC-IC experience. Anatomical measures that show EC-IC effects include differential cell counts [6], density of dendritic spines [12], dendritic branching [13] and synaptic dimensions [16,27].

Since Brown measured enzymatic activity only in occipital cortex, one might have supposed that the enduring behavioral effects were mediated by persistence of enzymatic differences in other brain regions after differences had disappeared in occipital cortex. Comparison of persistence of enzymatic effects in several brain regions (Tables 3 and 4 and Fig. 2), indicates that the effects last as long in the occipital cortex as elsewhere in the brain and that many regions show effects that persist for at least 47 days.

It is true that we did not measure persistence after Brown's version of environmental enrichment (IVS). We did, however, determine that EC and IVS yield closely comparable effects in brain weights and in AChE and ChE activities during a four-week-long experiment. The IVS treatment did not produce the extremely large effects in AChE and ChE activities reported by Brown.

The persistence of the induced brain effects is important in considering the possibility that some of them may either be involved in storage of memory for the differential experience or may reflect processes of memory storage. Thus, Brown [3] concluded that the fact that the enzymatic changes "... do not persist as long as the behavioral changes ... is inconsistent with a postulated cholinergic involvement in a purely quantitative manner in long-term storage and retrieval (p. 415)." Our finding that the enzymatic changes do, in fact, persist for weeks shows that one cannot rule out the possibility that these changes might be involved in memory storage. Deutsch [5] has provided completely independent evidence linking cholinergic synaptic modifications to memory storage. While we are not advocating the hypothesis that cholinergic changes store long-term memory, we do not believe that this hypothesis is ruled out by Brown's failure to find persistence of changes for more than a few days. It may be oversimple to expect a direct and linear relationship between a brain change that stores memory and any particular behavioral index of memory. For one thing, effects of early experience do not have uniform or equal effects on different tests of learning or problem-solving behavior. Prior enriched experience leads to superior performance on some tests, no changes on others, and impaired performance on still other tests [18]. Obviously, no brain measure could show the same relation to scores on behavioral tests that yield such different results. Furthermore, as we saw above, various brain measures show different rates of decline of effects, so that a failure to find a significant difference in one brain measure at a given point in time does not prove that no difference exists in another measure.

In certain previous papers we have called attention to "transitory components of the cerebral changes induced by experience" [2, 17, 21, 22]. By this was meant that as animals are kept in the EC or IC environments for prolonged periods, "Some of the brain weight differences pass through a maximum and then decrease ..." ([22] p. 237). We never reported the EC-IC weight differences disappear with prolonged exposure. In fact, we proposed "... that the changes that wax and wane during training may be correlates of the process of consolidation of memory, while some of the changes that endure may be correlates of the storage of memory" ([17] p. 61). Our only report of disappearance of an EC-IC effect with prolonged exposure has been that cortical depth effects were significant in motor, somesthetic, and occipital cortex in experiments run from 25 to 55 days of age but only in motor and occipital cortex in 25-to-105-day experiments; that is, the depth effect in somesthetic cortex became non-significant with the longer duration [7]. Unfortunately some readers seized on the term "transitory" and took it to apply to all of the EC-IC differences. We have reported highly significant brain weight differences after rats remained in EC or IC for 160 days ([22] p. 235); others have recently reported significant brain weight effects after a 530-day EC-IC period [4]. While those studies reveal that effects endure within the EC-IC period, the present paper demonstrates that the effects also persist for many weeks even after rats are removed from EC and placed in IC.

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