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Authors
Rosenzweig, Mark R.
Bennett, Edward L.

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Mark R. Rosenzweig
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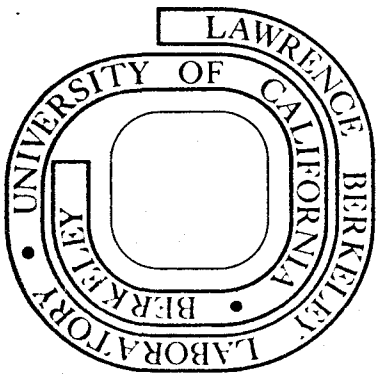

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CEREBRAL CHANGES IN RATS EXPOSED INDIVIDUALLY TO AN ENRICHED ENVIRONMENT[1]

MARK R. ROSENZWEIG[2] AND EDWARD L. BENNETT

This study is part of a continuing attempt to define the environmental conditions necessary to bring about cerebral differences between rodents exposed to enriched and impoverished environments. Rats placed singly in large enriched-environment cages for 2 hr. per day over a 30-day period were found to develop differences from home-cage control littermates in weights of brain sections and in brain-enzyme activities. These differences are small, however, unless the rat's interaction with the stimulus objects is facilitated; such priming can be accomplished by putting the rat in the enriched environment in the dark under the influence of an excitant drug. Social stimulation, which heretofore has always been included in the enriched condition, is now found not to be necessary.

A number of investigators have reported that giving a group of rodents prolonged informal experience in an enriched environment produces significant changes in chemical and anatomical brain measures (e.g., Altman & Das, 1964; Bennett, Diamond, Krech, & Rosenzweig, 1964; Brown & King, 1971; Diamond, Law, Rhodes, Lindner, Rosenzweig, Krech, & Bennett, 1966; Henderson, 1970; Krech, Rosenzweig, & Bennett, 1960; La Torre, 1968; Møllgaard, Diamond, Bennett, Rosenzweig, & Lindner, 1971; Rosenzweig & Bennett, 1969; Rosenzweig, Bennett, & Diamond, 1972a; Rosenzweig, Krech, Bennett, & Diamond, 1962; Walsh, Budtz-Olsen, Penny, & Cummins, 1969). Until now, the respective roles of the social grouping and of the enriched inanimate environment have not been determined, and doing so is the purpose of this study.

A prior attempt to resolve this question was presented in 1969 (Rosenzweig, 1971). The results indicated that neither social grouping by itself (in a simple environment) nor an enriched-stimulus situation given to individual animals produced clear cerebral effects. Two hypotheses were offered to account for these results: Either (*a*) the combination of social grouping and a complex inanimate environment is necessary to produce the brain changes, or (*b*) the complex environment would be sufficient to induce the brain effects *if* individual animals could be made to interact sufficiently with the environment. The second hypothesis was prompted in part by the observation that in the enriched environment an individual rat is active only about half of a 2-hr. period, whereas in a group of 12, each rat is active about three-quarters of the time. The following observation was a further indication that social behavior may not be important in this regard and that active exploration of the stimulus objects seems largely to determine the cerebral effects of environmental enrichment: Chang (1969) found that methamphetamine, which augments the cerebral effects of enriched experience (Rosenzweig & Bennett, 1968), reduces social behavior in the complex environment almost to zero while significantly increasing exploratory behavior. In order to test the second hypothesis, we decided to "prime" the activity of single rats, either by placing them in the enriched environment in the dark phase of the daily circle or by giving them a small dose of an excitant drug, or by doing both.

METHOD

Experimental Conditions

Rats were placed singly for 2 hr. per day over a 30-day period in the large enriched-environment cages that have been used as the standard enriched condition with grouped animals. Since the enriched environment with grouped animals has been called the enriched condition (EC) in many papers, a special designation is required for the single animal in the enriched environment; it will be called

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[2] Requests for reprints should be sent to Mark R. Rosenzweig, Department of Psychology, University of California, Berkely, California 94720.

single EC (SEC). Two hours per day in EC for 30 days has been shown to be sufficient to produce clear cerebral effects in grouped rats (Rosenzweig, Love, & Bennett, 1968). Furthermore, a 2-hr. period can readily be scheduled in different parts of the daily light–dark cycle, and 2 hr. of increased activity can be obtained with a single small dose of methamphetamine. Using this drug, we have already found enhanced cerebral effects with grouped rats (Rosenzweig & Bennett, 1968).

The six experimental conditions are shown in Table 1. All animals were housed in individual colony cages (32 × 20 × 20 cm.) in the same room. Lights were turned on by a timer at about midnight, and they went off at about noon; the exact schedule changed somewhat from experiment to experiment for convenience. During the dark part of the cycle, dim red lights were on to allow the experimenters to handle the rats and to give them injections. Home-cage rats received an injection each day but were not handled otherwise except for weighing every third day. In each of the four SEC conditions, the animals were injected in the room in which the home cages were placed. Upon injection, the rat was taken through an open doorway and placed in one of the 12 EC cages in the adjoining room. Groups c and d were run during the light period, c being run first on alternating days. Groups e and f were run in the dark, each being run first every other day.

Our standard EC cages were used (70 × 70 × 46 cm.). Each of the 12 cages had a different arrangement of about 6 stimulus objects, most of them from our standard pool of 25 objects (Rosenzweig & Bennett, 1969) and a few other objects also were used. Each rat was placed in a different EC cage each day in rotation, and new arrangements were put into all 12 cages every thirteenth day.

Injections

Each rat received an intraperitoneal injection of .003 ml/gm body weight each day. According to the group, the injection was either physiological saline or 2 mg/kg dextro desoxyephedrine hydrochloride (methamphetamine) in saline.

Subjects

The subjects were 275 male rats of the Berkeley S_1 line. A littermate design was employed in order to control for variability among subjects. Since few litters have six males, not all of the six conditions could be run at a time. Condition a, home cage (HC)–saline, was included as the base line in every experiment. From 10 to 12 litters containing three or more males with similar body weights were chosen for each experiment. Littermates were then assigned semirandomly among a number of groups equal to the conditions of an experiment, the only restriction being that the weight distribution be similar among groups. The groups were then assigned at random to the experimental conditions.

Within a few days after weaning, the animals were assigned to conditions, housed in individual cages, and placed on the altered light cycle. After about 1 wk. under these conditions, injections and daily SEC were begun.

TABLE 1

THE SIX EXPERIMENTAL CONDITIONS

Injection	In home cage 24 hr/day	In EC cage 2 hr/day	
		During light hours	During dark hours
Saline	a	c	e
Methamphetamine	b	d	f

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 (Rosenzweig et al., 1962). 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 could be combined to give total cortex; measures from the two remaining sections could be combined to give rest of brain (or subcortex).

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 (1961) has been adapted for the differential assay of acetylcholinesterase (AChE) and cholinesterase (ChE). In this procedure, thiocholine, which is the product of esterase activity on acetylthiocholine (AcSCh) or on butyrylthiocholine (BuSCh), reacts rapidly with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) to produce 5-thio-2-nitrobenzoic acid. At pH 8 this product is a highly colored anion and its rate of appearance can be determined readily and accurately by a suitable recording spectrophotometer. We have used this method with only minor modifications since 1963 and have described our procedure in detail in mimeographed notes that have been made available to other investigators since 1964 and can be obtained from the authors upon request.

Frozen brain samples are homogenized using a

commercial glass homogenizer with a Teflon pestle in cold .1 M sodium phosphate buffer (pH 8.0, 37° C.) and diluted to a final concentration of 3.0–5.0 mg/ml, depending upon the brain section. To determine AChE activity, the rate of hydrolysis of 6×10^{-4} M AcSCh is determined in the presence of 1.6×10^{-4} M DTNB and 2.5×10^{-5} M promethazine·HCl. The amount of brain tissue used depends upon the AChE activity of the section analyzed and ranges from .5-mg/3.0-ml of incubation mixture for a subcortical brain sample to 3.0 mg. for occipital cortex. The reactions are run at 37° C. in .1 M sodium phosphate buffer, pH 8.0. Promethazine·HCl is a selective inhibitor of ChE and at the concentration employed inhibits ChE approximately 70% while inhibiting AChE less than 5%. To determine AChE, Bayliss and Todrick (1956) and Klingman, Klingman, and Poliszczuk (1968) used ethopropazine as the selection inhibitor of ChE. Ethopropazine is very similar in structure and properties to promethazine which we began to use as a substitute when we could not obtain ethopropazine.

To determine ChE activity, the rate at which the brain homogenate hydrolyzes 1×10^{-3} M BuSCh is measured in the presence of 5×10^{-7} M 1,5-bis (N-allyl-N,N-dimethyl-4-ammonium phenyl) pentan-3-one dibromide (BW 284C51). At this concentration of BW 284C51, we have estimated that 98% inhibition of AChE activity is obtained, whereas ChE activity is inhibited less than 5%. These inhibition values agree with the reports of Bayliss and Todrick (1956) and Klingman et al. (1968). Since ChE activity is low in the rat brain, approximately 10 mg. of tissue are used in each 3.0 ml. of incubation mixture.

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 Weights

Each of the other groups was found to differ in some brain measures from the base-line HC–saline group, and the differences were largest and most consistent between the base-line animals and those given methamphetamine and put into EC in the dark hours. Main results for brain weights and terminal body weights are given in Table 2.[3] Our usual finding in comparing EC vs. an impoverished condition (IC or HC) is that the largest percentage effect occurs in the occipital cortex; total cortex and the ratio of total cortex to the rest of the brain both give very stable effects. Table 2 shows that for rats run individually the differences also tend to be largest in the occipital area and most significant for total cortex and the cortical/subcortical ratio.

Note in the overall data at the bottom of the table that even Group c—animals put into SEC in daylight hours after saline injection—differ significantly from the base-line group. The differences are small, however, and most do not show up consistently in individual experiments. This is why in previous experiments we concluded that rats put singly into EC did not develop brain effects. However, the difference in cortical/subcortical ratio is significant in two experiments ($p < .05$, $p < .01$) and in the same direction in two others, and this yields a highly significant overall difference ($p < .001$). The present results show that even single rats in EC yield effects, but these effects are clearly smaller than results obtained with rats placed in EC in groups for either 2 hr. or 24 hr. per day (see Table 3).

Putting rats into SEC in their dark hours when they are somewhat more active (Group e, dark–saline) did yield slightly larger effects than were obtained with Group c (light–saline), but none of the differences between e and c were statistically significant. Thus giving the enriched experience during the dark phase of the daily cycle was not much more effective than giving the experience during the light phase.

Putting rats in SEC under a light dose of methamphetamine proved to be highly effective. The dark–drug condition (f) was employed in five experiments and, as shown in the right-hand column of Table 2, each

[3] To conserve space, results are not given for all of the regions into which the brain was dissected, and certain regions have been combined. A detailed table giving means and standard deviations as well as percentage values for each region and each experiment is available, upon request, from the first author.

TABLE 2

EFFECTS OF ENVIRONMENT AND DRUGS ON BRAIN WEIGHTS AND BODY WEIGHTS

Experiment	*n* per group	Sacrifice date	*M* weights for HC–Saline base line (Group a)	% differences of brain and body weights[a]				
					2 hr. daily in large furnished cage			
					Light hours		Dark hours	
				HC–Drug (Group b)	Saline (Group c)	Drug (Group d)	Saline (Group e)	Drug (Group f)
Experiment 1	12	2/20/70						
Occipital cortex			65		8.5*		10.1***	14.0***
Total cortex			623		1.2		3.0*	7.3***
Rest of brain			821		−.6		.1	1.9
Cortex/Rest			.759		1.8*		2.8**	5.2***
Body weight			210		−4.4		−1.7	2.1
Experiment 2A	10	4/8/70						
Occipital cortex			69	−.1	2.5	4.8*		
Total cortex			622	1.7	1.6	4.0*		
Rest of brain			805	.9	.6	2.2		
Cortex/Rest			.774	.8	1.0	1.8		
Body weight			179	−3.0	1.4	4.9		
Experiment 2B	10	4/8/70						
Occipital cortex			70	.5			2.8	9.5***
Total cortex			620	3.4			4.4	6.7*
Rest of brain			808	1.0			1.9	3.6
Cortex/Rest			.768	2.3			2.4**	3.0*
Body weight			175	3.1			3.9	9.0
Experiment 3	12	6/11/70						
Occipital cortex			73	.3	−.1		1.0	7.8**
Total cortex			646	1.6	2.2		1.2	3.6
Rest of brain			842	1.4	.2		−.9	1.0
Cortex/Rest			.768	.2	2.0**		2.1*	2.6*
Body weight			212	2.1	6.8		.6	2.7
Experiment 4	12	8/20/70						
Occipital cortex			67	1.8	5.4*		4.0	11.1**
Total cortex			656	1.2	3.6*		3.6**	4.7**
Rest of brain			864	0.0	1.8		1.6	2.0
Cortex/Rest			.760	1.2	1.7		2.0**	2.6*
Body weight			209	−3.1	1.9		−1.9	4.9
Experiment 5	9	11/2/70						
Occipital cortex			69			10.1***		9.3**
Total cortex			628			3.4		3.8
Rest of brain			826			2.7		.6
Cortex/Rest			.761			.6		3.0*
Body weight			191			−2.4		−9.3
Overall								
Occipital cortex			69	.6	4.0**	7.3***	4.4***	10.4***
Total cortex			634	1.9*	2.2**	3.8*	3.0***	5.2***
Rest of brain			829	.8	.6	2.4	.6	1.8*
Cortex/Rest			.765	1.1**	1.6***	1.2	2.3***	3.3***
Body weight			198	.3	1.4	1.3	−.1	2.2

Note.—Brain weights are given in milligrams; body weights, in grams. Overall *n* for Group a = 65, Group b = 44, Group c = 46, Group d = 19, Group e = 46, and Group f = 55.

[a] Measured from base line of HC–Saline group.

* $p < .05$.

** $p < .01$.

*** $p < .001$.

TABLE 3

EC–IC Percentage Differences in Brain Weights and Terminal Body Weights for S_1 Rats Placed in EC in Groups

Weight measure	EC 24 hr/day[a]	EC 2 hr/day[b]
Occipital cortex	10.4**	11.0**
Total cortex	5.9**	3.9**
Rest of brain	.5	.5
Total brain	2.8**	2.0*
Cortex/Rest	5.3**	3.4**
Body weight	−11.2**	−2.5

[a] n = 135 pairs.
[b] n = 30 pairs.
* $p < .01$.
** $p < .001$.

experiment yielded highly significant differences from the HC–saline littermates. The light–drug condition (d) was tested only twice, and both experiments yielded significant effects in the occipital cortex but not in other brain regions. Because only one experiment included both a light– and a dark–drug–SEC group, we cannot be certain that light–drug SEC is less effective than dark–drug SEC. With methamphetamine given in the dark hours, SEC rats develop as large brain weight changes as do EC animals without drugs. (See the results for grouped rats in Table 3.)

Since the SEC–drug conditions produce such clear effects, it is necessary to ask how effective the drug itself may be even without the enriched environment. It was to provide this information that the HC–drug condition, b, was included in most experiments. Table 2 reveals that experiencing the drug in HC produced only slight effects that were rarely significant in a single experiment and that reached overall significance only for total cortex ($p < .05$) and for the weight ratio of total cortex to rest of brain ($p < .01$). The effects for Condition b were uniformly the weakest of any of the experimental conditions. Furthermore, statistical tests demonstrated the SEC–drug conditions to produce significantly larger brain effects than HC–drug conditions. Thus the large brain changes in the SEC–drug conditions are not attributable to the drug alone but to an addition or interaction of drug and environmental effects.

Comparison between the EC–drug and EC–saline groups showed that the effects in the drug groups are larger than those in the saline groups for each brain region. This is most clearly the case in the occipital cortex where the SEC–drug effects are twice as large as the comparable SEC–saline effects; the occipital cortex effect in the dark–SEC–drug groups is significantly greater ($p < .001$) than in the dark–SEC–saline group.

Note that differences in body weights among groups occurred irregularly and rarely reached statistical significance. In no case do the overall statistics at the bottom of Table 2 show a significant difference in body weights. Thus the effects in brain weights are not due to differences of conditions on body weights. Although amphetamines have been used to control appetite, daily administration, during 1 mo., of the small dose employed in these experiments had no effect on body weight. It should also be noted that although rats put into EC for 24 hr. per day at weaning characteristically lose weight compared to IC littermates (see the bottom row of the left column of statistics in Table 3), there is no such weight loss for 2-hr. daily EC (the right column in Table 3) or for 2-hr. daily SEC.

Chemical Measures

We have found previously that 80 days of exposure to EC vs. IC produces significant differences in activities of acetylcholinesterase (AChE) and of cholinesterase (ChE) in brain regions (Rosenzweig, Bennett, & Diamond, 1972b). The EC animals show a lower AChE activity per unit of cortical weight and higher ChE activity, as measured against IC littermates. In experiments of 30-day duration, while the AChE effects have already developed, the ChE effects have usually not yet emerged clearly. The present experiments reveal rather similar results in the case of SEC rats.

Chemical analyses were done on all experiments except the first. Analyses were lost for three of the rats. Table 4 presents overall results on AChE activity per unit of weight (see Footnote 3). The rats exposed to SEC in the dark or given methamphetamine (Groups d, e, and f) show signifi-

TABLE 4

EFFECTS OF ENVIRONMENT AND DRUG ON BRAIN ACETYLCHOLINESTERASE ACTIVITY

Experiment	AChE *M*s for HC–Saline base line[a] (Group a)	% differences in AChE activity[b]				
			2 hr. daily in large furnished cage			
			Light hours		Dark hours	
		HC–Methamphetamine (Group b)	Saline (Group c)	Methamphetamine (Group d)	Saline (Group e)	Methamphetamine (Group f)
Experiment 2A						
Occipital cortex	5.7	1.2	−.4	−3.9		
Total cortex	9.0	−.5	−1.5	−2.4		
Rest of brain	19.8	1.5	1.0	−.1		
Cortex/Rest	.456	−1.9	−2.4	−2.4*		
Experiment 2B						
Occipital cortex	5.6	−1.3			−3.1	−4.3
Total cortex	8.8	−1.0			−2.1	−2.2
Rest of brain	19.8	0.0			.1	−1.1
Cortex/Rest	.447	−1.0			−2.2	−2.2
Experiment 3						
Occipital cortex	5.8	−2.0	−4.3*		−3.9*	−4.1*
Total cortex	8.8	−1.2	−1.5		−2.1	−2.5
Rest of brain	19.3	.6	−.3		.4	−1.2
Cortex/Rest	.455	−1.8	−1.2		−2.5*	−1.4
Experiment 4						
Occipital cortex	5.7	−3.0	−1.6		−3.4	−2.7*
Total cortex	8.6	−2.8*	−1.8		−3.2*	−3.9*
Rest of brain	18.6	1.2	1.8		.8	.3
Cortex/Rest	.464	−3.9**	−3.5*		−3.9**	−4.2**
Experiment 5						
Occipital cortex	5.8			−5.2*		−4.2
Total cortex	9.0			−5.0**		−3.7**
Rest of brain	19.5			−1.2		−.4
Cortex/Rest	.463			−3.8**		−3.3**
Overall						
Occipital cortex	5.7	−1.4	−2.2	−4.0*	−3.5**	−3.8***
Total cortex	8.8	−1.4*	−1.6	−3.6***	−2.4*	−3.1***
Rest of brain	19.3	.8	.8	−.6	.5	−.6
Cortex/Rest	.457	−2.2***	−2.4**	−3.0***	−2.9***	−2.6***

Note.—Overall *n* for Group a = 52, Group b = 43, Group c = 34, Group d = 19, Group e = 33, and Group f = 42.

[a] AChE activity is expressed in units of nanomoles acetylthiocholine hydrolyzed/minute/milligram.

[b] Measured from base line of HC–Saline group.

* $p < .05$.

** $p < .01$.

*** $p < .001$.

cant reduction of AChE activity throughout the cortex and especially in the occipital region. Whereas for the brain weight measures, the dark–drug–SEC condition showed the largest effects, for AChE activity the magnitude of effects was quite similar among Groups d, e, and f. The rats put into SEC in the light hours and with a saline injection (Group c), however, developed only small AChE effects, only the drop in cortical/subcortical ratio being significant ($p < .01$).

Cholinesterase showed small but nonsignificant increases in activity in the cortex, in accordance with our previous findings that 30 days is not enough to produce ChE effects among rats exposed to EC in groups. The only significant ChE effect was an in-

crease in the cortical/subcortical ratio for the dark–drug group (1.3%, $p < .05$).

The ratio of ChE activity to AChE activity gives a purely chemical measure with tissue weights being cancelled out. This ratio of enzymatic activities affords a rather sensitive measure of EC–IC effects. Table 5 presents effects on ChE/AChE activities for the present experiments (see Footnote 3). All five experimental groups showed significant differences from the base-line HC–saline group in the cortical/subcortical ratio, as shown in the bottom row of Table 5. In addition, the groups given the drug or placed in SEC in the dark (Groups d, e, and f) also show significant effects in total cortex and even larger effects in occipital cortex.

In these enzymatic measures, as in brain weights, the individual rats primed to activity in the enriched environment develop brain differences from home-cage controls that are similar in magnitude to those of 30-day EC–IC experiments.

Discussion

The findings of this study help define further the range of conditions known to be necessary to produce the cerebral effects of environmental enrichment. Since the original demonstration that such effects could be produced by 80 days of highly differentiated conditions, the requirements have steadily been narrowed and made more explicit. Originally the enriched condition included not only about 23 hr. per day in the EC cage with varied stimulus objects, but an additional 30 min. in a Hebb-Williams open field with a pattern of barriers that was changed daily and also about ½ hr. of formal training. This condition was therefore called ECT–environmental complexity and training. The impoverished condition (IC) meant isolation in individual cages in a dimly illuminated area, and beginning in 1962 the IC animals were housed in cages with solid sidewalls in a separate room that was also quieter than the EC room.

We subsequently found that formal training added little or nothing to the cerebral effects (Rosenzweig, 1966), and further experiments showed that both the open-field experience and formal training could be omitted without reducing the effect (Rosenzweig, Love, & Bennett, 1968). Brown and King (1971) have reported that 80 days of exposure to varied stimulus objects is enough to produce significant effects in AChE and ChE activities in occipital cortex (no other measures were taken in their study). Brown's groups that were also given formal training did not show larger effects than those that had only informal experience. (We should note that we have reservations about the Brown study, both because of the low enzymatic activities reported and because of the surprisingly large differences in activities found between groups and the large variabilities within groups.)

The impoverished condition could be made less strict without reducing its effect, as the present experiment also demonstrates. When IC rats were housed in the same room as EC, the EC–IC differences were just as large as when measured against a littermate IC group housed concurrently in the dimly lighted, quiet IC room (Rosenzweig, Krech, Bennett, & Diamond, 1968). In the drug experiments, the HC or IC animals are handled and injected daily and are housed on the same racks with SEC rats, yet clear SEC–HC brain effects develop. In a recent control experiment, we assigned littermates to three impoverished conditions: (*a*) IC cages in the separate IC room, (*b*) HC conditions—single rats in colony cages in a room with normal lighting, (*c*) HC–injection—this is the same as *b*, except that the animals were also given a daily injection of physiological saline. After 30 days in these conditions, brain weight measures of all three groups were virtually identical. These results were why we did not hesitate to compare EC–IC results with SEC–HC results in Table 3.

The duration of differential experience could be reduced drastically and still yield many significant cerebral effects. When the duration of experiments was reduced from 80 to 30 days, the EC–IC differences in brain weights were even larger, since there is a transitory as well as an enduring component in the effects (Rosenzweig, Bennett,

TABLE 5

EFFECTS OF ENVIRONMENT AND DRUGS ON RATIO OF CHOLINESTERASE TO ACETYLCHOLINESTERASE ACTIVITY IN BRAIN

Experiment	*M* for HC–Saline base line[a] (Group a)	% differences in ChE/AChE activities[b]: HC–Methamphetamine (Group b)	2 hr. daily in large furnished cage: Light hours: Saline (Group c)	Light hours: Methamphetamine (Group d)	Dark hours: Saline (Group e)	Dark hours: Methamphetamine (Group f)
Experiment 2A						
Occipital cortex	5.390	0.0	3.5	6.6		
Total cortex	3.350	−.4	1.6	4.3		
Rest of brain	2.593	−2.0	−2.0*	−.2		
Cortex/Rest	1.292	1.7	3.6	4.6		
Experiment 2B						
Occipital cortex	5.489	1.8			4.9	5.8
Total cortex	3.352	.5			2.3	2.2
Rest of brain	2.610	−2.0			−2.1	−1.3
Cortex/Rest	1.286	2.4			4.3*	3.5
Experiment 3						
Occipital cortex	5.748	2.2	7.5*		6.4	5.8*
Total cortex	3.611	2.1	4.0*		3.9*	2.8
Rest of brain	2.954	−1.2	2.1		1.1	.7
Cortex/Rest	1.223	3.3*	1.9		2.8*	2.0
Experiment 4						
Occipital cortex	6.161	1.4	.1		−.5	1.8
Total cortex	3.652	2.8	−.7		1.6	2.3
Rest of brain	2.883	−.3	−3.5***		−1.6	3.0**
Cortex/Rest	1.267	3.1	3.0		3.3*	5.5**
Experiment 5						
Occipital cortex	5.261			8.4*		9.5***
Total cortex	3.364			6.4**		6.6**
Rest of brain	2.706			0.0		1.4
Cortex/Rest	1.244			6.4*		5.1**
Overall						
Occipital cortex	5.646	1.4	3.6	7.5**	3.4*	5.3***
Total cortex	3.483	1.4	1.6	5.3**	2.6**	3.3***
Rest of brain	2.766	−1.2*	−1.0	−.1	−.7	−.6
Cortex/Rest	1.261	2.7***	2.8*	5.4**	3.4***	4.0***

Note.—Overall n for Group a = 52, Group b = 43, Group c = 34, Group d = 19, Group e = 33, and Group f = 42.

[a] 10 × ChE activity/AChE activity.

[b] Measured from base line of HC–Saline group.

* $p < .05$.

** $p < .01$.

*** $p < .001$.

& Diamond, 1967); the differences in AChE/weight were clear after 30 days, but the differences in ChE/weight were only beginning to emerge (Rosenzweig et al., 1972b). The EC–IC differences in cortical thickness, originally found in 80-day experiments (Diamond, Krech, & Rosenzweig, 1964), are even larger in 30-day experiments (Diamond, Rosenzweig, Bennett, Lindner, & Lyon, 1972). The 30-day period was also effective in inducing in the gerbil differences in brain weights and enzymatic measures similar to the effects found in the rat (Rosenzweig & Bennett, 1969). Differences in synaptic measures in the rat cortex have also been found in 30-day experiments (Møllgaard et al., 1971). The experience can be reduced to 2 hr. per day over

a 30-day period, and EC–IC differences in brain weights and in AChE/weight are as large as with 24 hr. per day exposure (Rosenzweig, Love, & Bennett, 1968; Rosenzweig et al., 1972b).

Are the cerebral effects induced by the drug simply additive with the effects of the enriched environment, or do the drug and environmental conditions interact in producing the effects? Only in the case of weight of occipital cortex is there clear evidence of interaction. Here Table 2 shows the effect of the drug to be .6% (the difference between the HC–saline and HC–drug conditions) and the effect of the dark–SEC–saline condition to be 4.4%; thus the additive result of drug and dark SEC would be 5%, whereas the dark–SEC–drug condition was actually much larger—10.4%. For other measures, the result appears to be essentially additive. For example, in the case of weight of total cortex, the addition of the drug and dark SEC effects is 4.9%, whereas the actual difference between HC–saline and dark–SEC drug was 5.2%. Why interaction occurs only in the occipital area is not clear. This is the only area that yields effects as large as 10% and the difference between additive and interactive combinations may be shown most clearly when effects are relatively large. It is also possible that there is a ceiling or maximum to certain effects, and this may be why even an additive effect does not appear. For example, consider AChE activity per unit of weight. Here Table 4 showed the effect of the drug on AChE in occipital cortex to be −1.4% and the effect of the dark–SEC–saline condition to be −3.5%; thus the additive effect would be −4.9%, whereas the result for the dark–SEC–drug condition was only −3.8%. For the cortical/subcortical ratio of AChE the dark–SEC–drug result (−2.6%) was not quite as large as either the dark–SEC–saline effect (−2.9%) or the light–SEC–drug effect (−3.0%). This suggests that either factor (drug or SEC) could induce the full effect and their combination could accomplish no more. Thus, depending upon the measure to be employed and the brain region considered, the combination of drug and enriched environment may yield an effect that (*a*) does not exceed that of either of the two factors, that (*b*) appears to be the sum of the effects of the two factors operating separately, or that (*c*) is clearly larger than the sum of the separate effects of the two factors.

From this and other studies, we hypothesize that anatomical and chemical changes will develop in the rodent brain whenever the animal interacts with a relatively complex environment for at least a minimum daily period (perhaps 1 hr.) over at least a minimum duration (perhaps a few weeks). The brain is clearly more plastic than was thought a decade ago. But certain minimum conditions must be fulfilled in order to induce changes of several percent in brain weights and brain enzyme activity. Putting a group of rats in a large empty cage has little effect, and putting a single rat in a complex environment has only a small effect *unless* the rat is primed to interact with the stimulus objects. The most effective condition that we have found so far for this purpose is giving a small dose of methamphetamine to a rat before placing it in SEC during the dark part of the daily cycle. Further tests of this hypothesis are to be run, employing other means to encourage interaction with the complex environment.

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