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Environmental Enrichment and Neurotransmitter Receptors¹

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The extent of high affinity, specific binding of several pharmacological agents to brain membrane fractions derived from rats reared in an environmentally enriched or impoverished environment has been assayed. The binding of all labeled agents studied was not significantly altered in cerebellar, subcortical, or cortical membranes by the rearing condition. In the case of cortical membranes, binding of ³H-benzodiazepine, ³H-dihydroalprenolol, ³H-dihydroergocryptine, ³H-spiroperidol, ³H-quinuclidinyl benzilate (QNB), and ³H-muscimol was not detectably altered by exposure to these two housing conditions. These data suggest that the observed small but statistically significant increase in density of cortical synapses in rats kept in the "enriched" condition may not necessarily reflect a great increase in a specific class of synapse.

The effect of experience on the physical and chemical characteristics of the brain has been the subject of many studies, and several reports have suggested that the environment may modulate cerebral anatomy and biochemistry (Rosenzweig & Bennett, 1976; Dunn, 1976; Greenough, 1976).

Over a period of 20 years, Rosenzweig and co-workers have consistently demonstrated a relation between experiential enrichment and several chemical and morphological parameters of the rat cortex (Bennett, 1976; Rosenzweig & Bennett, 1977). Some factors that have been repeatedly and rather consistently shown to be influenced by the housing conditions of experimental animals include cortex weight and its content of macromolecules (protein, DNA, RNA). Certain enzymes related to

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brain function such as acetylcholinesterase have also been shown to be modified by the environment (Rosenzweig, Bennett, & Diamond, 1972). Many chemical and morphological changes have been delineated in some detail and supportive data has come from several laboratories (Altman & Das, 1964; Volkmar & Greenough, 1972; La Torre, 1968; Grouse, Schrier, Bennett, Rosenzweig, & Nelson, 1978).

The mechanisms underlying such changes are unknown. Various possible means by which the environment may modulate brain composition have been put forward. These include the concept of an endocrine basis, perhaps stress related, for such differences. This possibility has been largely excluded, as have other factors present in these experimental conditions such as differences in social stimulation, locomotor activity, handling, or earlier maturation (Rosenzweig & Bennett, 1977). Recent findings have supported the original hypothesis in these studies that the cerebral changes are caused by the increased opportunity for learning that animals have when they are maintained in the enriched situation (Bennett, Rosenzweig, Morimoto, & Hebert, 1979). The metabolic processes mediating learning and biochemical changes in the brain are also uncertain but may be related to dynamic physiological changes connected with the use or disuse of specific neuronal circuits.

While the causes of the dependence of brain chemistry upon the external environment are not well understood, such chemical changes have been shown to be concurrent with specific cerebral anatomical variations. Thus, rats exposed to the environmentally enriched (EC) paradigm delineated by Bennett, Diamond, Krech, and Rosenzweig (1964) have increased cortical depth relative to animals maintained in the environmentally impoverished (IC) housing situation (Bennett, 1976; Rosenzweig et al., 1972; Diamond, Lindner, Johnson, Bennett, & Rosenzweig, 1975).

These changes appear confined to certain cortical regions, as do differences in size of neuronal perikarya and nuclei (Rosenzweig et al., 1972) and glial cell number (Diamond, Law, Rhodes, Lindner, Rosenzweig, Krech, & Bennett, 1966). Other anatomical measures that seem in part environmentally regulated are the density of dendritic spines and extent of dendritic arborization in the rat cortex (Volkmar & Greenough, 1972; Globus, Rosenzweig, Bennett, & Diamond, 1973). The concept underlying the present work was the possibility that the increased number and density of dendritic spines and size of receptor areas observed in EC rats may reflect a difference in the number of cortical neurotransmitter binding sites.

METHODS

Housing Conditions

Male rats of the Berkeley S₁ strain were used. This strain is derived from a Tryon maze bright stock. After weaning, paired littermates were

housed in enriched conditions (EC) or in an impoverished sensory condition (IC). Standard colony (SC) rats were reared in an intermediate environment (Bennett, 1976; Bennett & Rosenzweig, 1981).

In brief, EC animals were maintained in groups of 10 to 12 in a large cage provided with stimulus objects (ladders, wheels, etc.). IC animals were maintained singly in a dimly illuminated room in cages with solid side walls so that rats could not see each other. For the SC condition, three animals of the same sex were housed in each cage, measuring $21 \times 34 \times 20$ cm, made of wire bars with shavings on the floor (Bennett & Rosenzweig, 1981). This housing condition resembles that used by most laboratories that maintain rats. Food and water were freely available to all groups. After 35 to 55 days of exposure to these conditions, animals were killed and the cerebral cortex and subcortical regions were dissected out, weighed, and labeled by a coding system (Bennett & Rosenzweig, 1981). Subcortex included thalamus, hippocampus, and hypothalamus but excluded pons, medulla, and cerebellum. Storage before biochemical processing was between -20°C and -60°C .

Membrane Preparation

A crude membrane fraction was prepared from tissue by homogenization in 19 volumes 0.32 M sucrose and centrifugation ($50,000g$ for 10 min). The resulting precipitate was homogenized in the same volume of 40 mM Tris-HCl, pH 7.4, and recentrifuged ($50,000g$ for 10 min). This procedure combined with the prior freezing of brain parts caused major lysis of cell components such as mitochondria and nerve endings. In the estimation of GABA and diazepam binding, two further washes with Tris buffer were necessary in order to remove an endogenous inhibitory material.

Binding Assay

The use of the binding technique in the assay of neurotransmitter receptors has been extensively described (Yamamura, Enna & Kuhar, 1978). Binding measurements were performed in a 1 ml incubation volume containing 40 mM Tris, pH 7.4, and appropriate radioactive and unlabeled pharmacological agents. Incubation was at 37°C for 10 min, except in the case of assay for GABA and diazepam receptors when a temperature of 2°C was used. The amount of membrane tissue in each incubation corresponded to 5 mg original wet weight and contained around 300–400 μg protein as determined by the method of Lowry, Rosenbrough, Farr, and Randall (1951).

A series of preliminary studies was carried out (Bondy, 1980) establishing that binding was at equilibrium within 10 min, was saturable and generally reversible, was between 75 and 95% specific, had an appropriate

regional distribution, and was limited by and proportional to the amount of membrane present.

Additional studies delineated the specificity of ligand binding (Agrawal & Bondy, 1981). The concentration of labeled ligand was sufficiently low to ensure that high-affinity interactions would predominate. Final concentrations of labeled compounds were 1.0 nM for (1-phenyl-4-³H)-spiroperidol (25.6 Ci/mmol); 1.3 nM for 9,10-³H-dihydroergocryptine (21 Ci/mmol); 0.7 nM for 1 propyl 2,3-³H dihydroalprenolol (40 Ci/mmol); 1.0 nM for DL-(benzyl 4-4-³H)-quinuclidinyl benzilate (QNB) (29.4 Ci/mmol); 1.0 nM for (methylene ³H(N)-muscimol (13.7 Ci/mmol); and 0.75 nM for (methyl-³H)-diazepam (64 Ci/mmol).

All determinations were in triplicate, and a triplicate series of tubes containing a large excess of an unlabeled competing ligand was simultaneously incubated in order to estimate nonspecific binding. The final concentrations of these competitors were 1 μ M haloperidol (for ³H-spiroperidol), 1 μ M ergocryptine (for ³H-dihydroergocryptine), 1 μ M alprenolol (for ³H-dihydroalprenolol), 10 μ M atropine (for ³H-QNB), 10 μ M GABA (for ³H-muscimol), and 3 μ M diazepam (for ³H-diazepam).

At the end of incubation, samples were filtered on glass fiber disks (25 mm diameter, 0.3- μ m pore size, Gelman, Ann Arbor, Mich.) and washed twice rapidly with 10 ml Tris-HCl, pH 7.4, at 0°C. Filters were then dried and counted in 5 ml of a scintillation mixture (Aquasol, New England Nuclear Corp., Boston, Mass.) using a scintillation counter at an efficiency of 38 to 43%.

Statistics

All data presented are based on results from nine pairs of littermates, one of each pair being assigned to the EC or IC condition. Each region from each animal was individually assayed. Unpaired data were evaluated using Students' two-tailed *t* test. Paired data were subjected to the correlated *t* test for matched pairs.

RESULTS AND DISCUSSION

Cortical weights of EC animals were around 5% heavier (666 ± 11 mg) than those of IC animals (634 ± 14 mg). In 14 out of 15 cases, the EC cortical weight was greater than that of the paired IC littermate. The extent of binding of six ligands by membranes prepared from cortical tissue was measured (Table 1). No significant differences were found between receptor binding of EC and IC animals on a protein basis for any of the six receptors assayed. Homologous measurements were also made using membranes prepared from the cerebellum or subcortical regions. Very little difference between the binding capacity of homologous tissue derived from EC and IC rats was found for any ligand studied (Table 1), and none of these small differences was statistically significant.

TABLE 1
Specific Binding of Labeled Compounds to Membranes of Rats Maintained in an Enriched (EC) or Impoverished (IC) Environment^a

Labeled compound	Putative binding site	Cortex			Subcortex			Cerebellum		
		EC	IC	EC/IC	EC	IC	EC/IC	EC	IC	EC/IC
Spiroperidol	Dopamine/ serotonin	98 ± 11	94 ± 9	1.05	153 ± 6	151 ± 9	1.02	—	—	—
Dihydroergocryptine	α-Adrenergic	199 ± 14	192 ± 10	1.04	196 ± 10	201 ± 9	0.98	—	—	—
Dihydroalprenolol	β-Adrenergic	65 ± 4	60 ± 4	1.09	47 ± 2	50 ± 4	0.95	36 ± 1	33 ± 2	1.11
QNB	Muscarinic cholinergic	840 ± 9	822 ± 25	1.02	711 ± 44	754 ± 43	0.94	87 ± 3	88 ± 3	0.99
Muscimol	GABA	81 ± 5	81 ± 3	1.00	42 ± 3	43 ± 2	0.98	124 ± 9	116 ± 9	1.07
Diazepam	Benzodiazepine	94 ± 8		0.97	79 ± 4	82 ± 5	0.96	41 ± 5	46 ± 3	0.88

^a *N* = 12 for each group. Data are expressed as pmoles ligand bound/g protein. Standard errors are presented. Experimental details are given in the text. Animals were maintained for 35 days in the two environmental conditions.

TABLE 2
Specific Binding of Labeled Compounds to Membranes from Brain Regions of Rats Maintained in an Enriched (EC), Impoverished (IC), and Social Control (SC) Environment^a

Labeled compound	Striatum			Hypothalamus			Hippocampus		
	EC	SC	IC	EC	SC	IC	EC	SC	IC
QNB	1087 ± 47	1007 ± 49	952 ± 18	333 ± 12	355 ± 10	347 ± 16	554 ± 23	507 ± 60	546 ± 37
Serotonin	103 ± 5	105 ± 5	96 ± 2	—	—	—	48 ± 5	49 ± 6	51 ± 2
Spiroperidol	625 ± 39	546 ± 49	543 ± 14	—	—	—	120 ± 14	144 ± 15	132 ± 14
Dihydroergocryptine	—	—	—	151 ± 11	156 ± 16	164 ± 18	—	—	—

^a Six to nine animals were used in each group. Data are expressed as pmoles ligand bound/g protein. Standard errors are presented. Experimental details are given in the text. Animals were maintained for 55 days in the three environmental conditions.

Four receptor sites were also measured in the cerebellum (the remaining two, dopamine and α -norepinephrine, receptors being too low to detect). No significant differences were apparent between data from EC and IC animals (Table 1). However, since EC animals had heavier cortices containing more protein than the corresponding IC animals, it follows that the overall binding capacity of EC cortices was greater than that of the IC cortices. It is interesting that EC/IC values for almost all neurotransmitter receptors studied were above unity in cortical and below unity in subcortical tissues. This result is somewhat reminiscent of the finding of increased cortical weight relative to subcortex in EC-treated rats (Rosenzweig et al., 1972).

Since certain subcortical regions may be sensitive to environmental modulation (Uphouse, 1980), receptor binding was studied in several more defined noncortical regions (Table 2). These regions were studied in more detail in order to increase the probability of detecting a region-specific change in a receptor-ligand interaction. In this study, social control (SC) animals were also studied. Fewer isotopes were used in this study due to relatively small weights of tissue being available. This, combined with the lower *N* value may account for the somewhat greater variances in Table 2. Striatal spiroperidol and hypothalamic ergocryptine binding were chosen, as binding of these ligands has been reported to be altered by prolonged isolation (Guisado et al., 1980) or by handling (Uphouse, 1981), respectively. However, no significant differences were found between the three experimental groups (Table 2).

In conclusion, although gross sensory deprivation is known to cause very selective changes of certain synapse types in precise anatomical areas (Winfield, 1981), we have found no evidence for an especial vulnerability of any single class of neurotransmitter specific neuron. However, it is possible that real effects were masked in these studies for two reasons. First, the standard errors of the results reported are sufficiently large such that changes of 5 to 10% could remain undetected in several cases. Second, the anatomical area studied was relatively large, and differences between smaller submicroscopic regions could have been obscured. The generally nonselective nature of the differences between animals raised in differing environments suggests that regional nutrition may play a role in effecting these differences. Changes in cerebral blood flow have been found to follow altered sensory input (Bondy & Davis, 1972). These dynamic metabolic events that alter nutrient supply could underlie the differences in chemical composition of the brains of animals maintained in environments of varying sensory potential.

REFERENCES

- Agrawal, A. K., & Bondy, S. C. (1981). Characterization of catecholamine binding sites in the mature rat brain. *Neurotoxicology*, (in press).
- Ahlenius, S. (1979). An analysis of behavioral effects produced by drug-induced changes

- of dopaminergic neurotransmission in the brain. *Scandinavian Journal of Psychology*, **20**, 59–64.
- Altman, J., & Das, G. P. (1964). Autoradiographic examination of the effects of enriched environment on the rate of glial multiplication in the adult rat brain. *Nature (London)*, **204**, 1161–1163.
- Bennett, E. L. (1976). Cerebral effects of differential experience and training. In M. R. Rosenzweig & E. L. Bennett (Eds.), *Neural Mechanisms of Learning and Memory*, pp. 279–287. Cambridge: MIT Press.
- Bennett, E. L., Diamond, M. C., Krech, D., & Rosenzweig, M. R. (1964). Chemical and anatomical plasticity of the brain. *Science*, **146**, 610–619.
- Bennett, E. L., Rosenzweig, M. R., Morimoto, H., & Hebert, M. (1979). Maze-training alters brain weights and critical RNA/DNA ratios. *Behavioral and Neural Biology*, **26**, 1–22.
- Bennett, E. L., & Rosenzweig, M. R. (1981). Behavioral and biochemical methods to study brain responses to environment and experience. *Methods in Neurobiology*, **2**, 101–141.
- Bondy, S. C. (1980). Rapid screening of neurotoxic agents by *in vivo* and *in vitro* means. In R. M. Gryder & V. H. Frankos (Eds.), *Effects of Food and Drugs on the Development and Function of the Nervous System*, pp. 133–143. Washington, D.C.: U.S. Govt. Printing Office.
- Bondy, S. C. (1981). Neurotransmitter binding interactions as a screen for neurotoxicity. In A. Vernadakis & K. N. Prasad (Eds.), *Mechanisms of Neurotoxic Substances*. New York: Raven Press.
- Bondy, S. C., & Davis, F. N. (1972). Cerebral circulation: Response to visual deprivation. *Brain Research*, **43**, 606–610.
- Bondy, S. C., & Harrington, M. E. (1978). Brain blood flow: Alteration by prior exposure to a learned task. *Science*, **199**, 318–319.
- Bondy, S. C., Lehman, R. A., & Purdy, J. L. (1974). Visual attention affects brain blood flow. *Nature (London)*, **248**, 440.
- Diamond, M. C., Lindner, B., Johnson, R., Bennett, E. L., & Rosenzweig, M. R. (1975). Differences in occipital cortical synapses from environmentally enriched, impoverished, and standard colony rats. *Journal of Neuroscience Research*, **1**, 109–119.
- Diamond, M. C., Law, F., Rhodes, H., Lindner, B., Rosenzweig, M. R., Krech, D., & Bennett, E. L. (1966). Increases in cortical depth and glial numbers in rats subject to enriched environment. *Journal of Comparative Neurology*, **128**, 117–125.
- Dunn, A. J. (1976). The chemistry of learning and the formation of memory. In W. H. Gispen (Ed.), *Molecular and Functional Neurobiology*, pp. 347–387. Amsterdam: Elsevier.
- Globus, A., Rosenzweig, M. R., Bennett, E. L., & Diamond, M. C. (1973). Effects of differential experience on dendritic spine counts. *Journal of Comparative and Physiological Psychology*, **82**, 175–181.
- Greenough, W. T. (1976). Enduring brain effects of differential experience and training. In M. R. Rosenzweig & E. L. Bennett (Eds.), *Learning and Memory*, pp. 255–278. Cambridge: MIT Press.
- Grouse, L. D., Schrier, B. K., Bennett, E. L., Rosenzweig, M. R., & Nelson, P. G. (1978). Sequence diversity studies of rat brain RNA: Effects of environmental complexity on rat brain RNA diversity. *Journal of Neurochemistry*, **30**, 191–203.
- Guisado, E., Fernandez, Tome P., Garzon, J., & del Rio, J. (1980). Increased dopamine receptor binding in the striatum of rats after long-term isolation. *European Journal of Pharmacology*, **65**, 463–464.
- La Torre, J. C. (1968). Effect of differential environmental enrichment on brain weight and on acetylcholinesterase and cholinesterase activities in mice. *Experimental Neurobiology*, **22**, 493–503.

- Lowry, O. H., Rosenbrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265-275.
- Rosenzweig, M. R., & Bennett, E. L. (1976). *Neural Mechanisms of Learning and Memory*. Cambridge: MIT Press.
- Rosenzweig, M. R., Bennett, E. L., & Diamond, M. C. (1972). Chemical and anatomical plasticity of the brain: Replications and extensions. In J. Gaito (Ed.), *Macromolecules and Behaviour*, 2nd ed., pp. 205-277. New York: Meredith.
- Rosenzweig, M. R., & Bennett, E. L. (1977). Effects of environmental enrichment or impoverishment on learning and on brain values in rodents. In A. Oliverio (Ed.), *Genetics, Environment, and Intelligence*, pp. 163-196. Amsterdam: Elsevier.
- Uphouse, L. L. (1980). Revaluation of mechanisms that mediate brain differences between enriched and impoverished animals. *Psychological Bulletin*, **88**, 215-232.
- Uphouse, L. L. (1981). Interactions between handling and acrylamide on the striatal dopamine receptor. *Brain Research*, **221**, 421-424.
- Volkmar, F. R., & Greenough, W. T. (1972). Rearing complexity affects branching of dendrites in the visual cortex of the rat. *Science*, **176**, 1145-1147.
- Winfield, D. A. (1981). The postnatal development of synapses in the visual cortex of the cat and the effects of eyelid closure. *Brain Research*, **206**, 166-171.
- Yamamura, H. I., Enna, S. J., & Kuhar, M. J. (1978). *Neurotransmitter Receptor Binding*. New York: Raven Press.