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EFFECTS OF ENVIRONMENTAL COMPLEXITY AND DEPRIVATION ON BRAIN ANATOMY AND HISTOLOGY: A REVIEW

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During the last 20 years the anatomical plasticity of the brain in response to sensory stimulation has been clearly demonstrated. This paper reviews the effects of environments rich in sensory stimulation versus those which are stimulus poor. Effects have been noted at all levels from the gross anatomical to the electromicroscopic.

As compared to their stimulus-deprived counterparts, animals reared in complex environments tend to display greater cerebral weight and length and cortical depth. The greatest effects occur in the occipital cortex where histological studies have revealed expanded neuron perikaryonal and nuclear size and dendritic branching, more dendritic spines, alterations in synaptic numbers and morphology, and greater numbers of neuroglia. Different cortical areas and layers respond to different degrees. Some responses have also been noted in the underlying hippocampus, particularly in the dentate gyrus. The emerging data present a picture of a dynamic, plastic brain adapting homeostatically to the demands of its environment.

Only within the last two decades has it been unequivocally demonstrated that the sensory environment in which an animal lives elicits detectable changes in brain structure. Contrary to previous beliefs it is now clear that the brain is a plastic organ which adapts to the demands of its environment across levels from whole brain size and weight down to ultra-structural components. Moreover, these responses are not global and non-specific but rather show regional, temporal, parameter, and stimulus specificity.

One particularly useful experimental paradigm for the investigation of environmental effects has compared the brains of animals reared in sensorially complex as opposed to deprived environments. A large number of anatomical differences has been detected and these are the subject of this paper.

Research in fields such as this tends to evolve through a sequence of stages. The first is essentially one of response detection in which attention focuses on detecting those parameters which show significant effects. This stage establishes the existence of a research field and allows the development of other stages which examine such factors as the temporal profiles of the responses, the nature of the

environmental stimuli which are effective in eliciting responses, and the nature of the psychological, physiological and chemical mechanisms which mediate the production of the observed effects.

Each of these investigations represents a sizeable research area. Relationships to functional and behavioral changes and possible mediating mechanisms have already been discussed elsewhere (Walsh & Cummins, 1975; Rosenzweig *et al.*, 1971; Jones & Smith, 1980; Rosenzweig, 1980; Walsh, 1980) as have chemical and physiological effects (Walsh, 1980), species differences (Walsh, 1980), and therapeutic implications (Walsh & Greenough, 1976). The present paper will therefore focus on a review and analysis of the responses themselves, examining their nature, magnitude and specificity, and within the current limits of knowledge attempting to assess their functional significance. To do this the nature of the complex and deprived environments will first be noted and then their effects on brain will be examined, starting first with the grosser anatomical parameters such as weight and cerebral size and moving to finer and finer levels.

METHODS OF DIFFERENTIAL REARING

The following description applies to the most common conditions which may, of course, vary from one experiment to another. Littermate rat pups, matched for body weight at weaning at about 25 days of age, are assigned to one of two, or less frequently three, environments. Environmental complexity (EC) is constituted by social groups of about 10 animals to whose cage toys are added and changed daily. (See Rosenzweig & Bennett, 1969 and Walsh, 1980 for photographs.)

Matched littermates are placed in smaller, solid-wall cages, either alone (isolated condition, IC) or sometimes in groups (social condition SC). Differential rearing continues for periods ranging from 4 to 900 days at which time the animals are sacrificed, the brains removed, and anatomical measures obtained.

NEUROANATOMICAL EFFECTS

Brain Weight

The effects of environmental complexity on whole brain weight are small and not easily found without careful measurement of large numbers of matched subjects. Unless otherwise specified the subjects mentioned are male rats differentially reared from weaning, the comparison is between EC and IC groups, and measurements were made blind.

In the Berkeley laboratories the effect on whole brain after 80 days of differential rearing amounted to a 1.0% increase significant only at the 0.01 level in spite of having run a total of 175 pairs (Rosenzweig *et al.*, 1971). Walsh *et al.* (1974), using over 200 pairs differentially reared for periods from 18 to 530 days, found a mean EC-IC difference of 2.6%. Significant findings have also been reported after 4 to 60 days by Ferchmin *et al.* (1970) and 30 days (Geller *et al.*, 1965). For the mouse, Henderson (1970) reported a 4.5% increase in EC compared with SC mice differentially reared for six to seven weeks from birth, though this effect was not detectable in any of six parental inbred strains but only in their F1 and F2 crosses. However, La Torre (1968) found increases of 5.7% and 4.5 in two inbred strains, and these differences are larger than those usually reported for the rat. Not surprisingly, several workers using smaller numbers of subjects, have been unsuccessful in

finding significant effects. The small size of the whole brain response is partly due to the regional localization of response. It should be noted that, although for ease of description, the effects are often labelled as due to complexity, this is an arbitrary perspective and it could just as arbitrarily and incorrectly be said that they are due to isolation. A more correct but lengthy statement would allude only to *differences* since all measures are relative and would claim only that EC-IC differential rearing elicits differences. This issue is discussed at length elsewhere (Rosenzweig *et al.*, 1978; Walsh, 1980).

Regional Specificity

The increase in weight on exposure to different environments is not uniform throughout the brain, but rather displays patterns of regional and temporal specificity. The terminology used to describe this regional localization bears only a partial relationship to the functional and anatomical nature of the areas but rather reflects the method of brain dissection evolved by the Berkeley workers. This dissection is performed with the aid of a plastic T square which is placed over the brain and whose markings guide a freehand dissection with which samples of occipital, somesthetic, and remaining dorsal cortex are peeled off from the underlying corpus callosum. Finally, all the remaining cerebral tissue is stripped from the underlying subcortex and named ventral cortex, though, in point of fact, it comprises not only ventral cortical tissue but also adjacent organs including amygdaloid nuclei, hippocampus, dentate gyrus and corpus callosum (Rosenzweig *et al.*, 1962). The remaining tissue, comprising olfactory bulbs, diencephalon, midbrain (mesencephalon), and hindbrain (rhombencephalon) is referred to as subcortex.

Within the cortex, effects after 80 days are maximal in the occipital region (6.4%), with the next most marked finding occurring in dorsal cortex (5.0%), then ventral (3.0%) and least (1.9%) in the somesthetic area. For the cortex as a whole (total cortex) this yields a mean effect of 4% which contrasts with a decrease of 1.1% in the remaining brain or subcortex (Rosenzweig *et al.*, 1971). Effects tend to be larger after only 30 days differential rearing (Rosenzweig & Bennett, 1978) or when even more complex environments are used (Kuenzle & Knusel, 1974; Rosenzweig & Bennett, 1978).

Since the cortical and subcortical effects are in opposite directions, the cortical: subcortical ratio (C/S) yields a larger and more sensitive effect (5.2%) than either alone (Zolman & Morimoto, 1962, 1965; Rosenzweig *et al.*, 1971). Why this should be so is unclear and it may be that several factors are involved. The opposite directions of change between cortex and subcortex increase the magnitude of the ratio effect and presumably reflect some regional patterning of neural activity, though the nature of this patterning is quite unclear at this time. A ratio measure between brain components has the advantage of removing much of the variance found in absolute nonratio measures attributable to individual variations in confounding variables such as genetic, somatic, nutritional, and endocrine factors. For the above reasons the C/S ratio may be expected to be not only more sensitive, but also to display less variability than either component alone.

Regional localization has not been so precisely delineated in the subcortex. No effect was found on total hindbrain weight (Ferchmin *et al.*, 1970; Walsh *et al.*, 1974) or on most hindbrain components (Rosenzweig *et al.*, 1962). Some discrete localization may exist since a significant difference in width was found in the area underlying the frontal (Walsh *et al.*, 1972) but not occipital (Diamond *et al.*, 1966) cortex, and in the mesencephalon both the superior and inferior colliculi have been found to be heavier after environmental complexity (Krech *et al.*, 1963). Floeter and Greenough (1978) have clearly demonstrated subcortical regional specificity at the neuronal level within the cerebellum. They noted purkinje cell dendritic effects within the paraflocculus and nodulus, but not in the flocculus. Whether other subcortical histological regional specificity can be detected remains to be determined.

It has frequently been suggested that extracellular fluid might be responsible for the observed weight changes. However, since both wet and dry measures yield the same order of effects (Bennett *et al.*, 1969; Eterovic & Ferchmin, 1974; Hoover & Diamond, 1976) it must be concluded that any differential fluid changes are minimal.

The Pineal

Though never empirically refuted, the pineal's original role as "the seat of the soul" has been largely disregarded and it is now thought to function more as a neuroendocrine transducer

particularly involved in reproductive physiology. Since a number of endocrine changes follow complexity-isolation rearing, e.g., pituitary, thyroid, adrenal, thymic, testicular, liver and splenic weights (Hatch *et al.*, 1963; Cummins, Note 2, Walsh *et al.*, 1971) it seemed possible that complexity might modify the pineal. However, in the only published report to date (Quay *et al.*, 1969) it was found that social density but not complexity *per se* altered pineal weight but neither factor influenced midday 5-hydroxytryptamine concentration or acetylserotonin methyltransferase activity. As yet the relative contributions of social and physical factors to the induction of the complexity-isolation endocrine differences listed above is unknown so it is impossible as yet to guess at the role of the pineal in their production. Further studies which separate the effects of these factors will be necessary before an answer can be given.

Cerebral Dimensions

The growth of the rat brain is not complete at birth nor yet at weaning but in fact continues throughout life, though only very slightly after about 75 days. In the cerebrum growth in width is virtually complete by 20 days but length continues to increase beyond 90 days (Bennett & Rosenzweig, 1968; Altman *et al.*, 1968b; Walsh *et al.*, 1971). This growth is susceptible to environmental stimulation since four months of operant conditioning, ten days of handling during infancy, and three months of environmental complexity were each found to increase cerebral length but not width (Altman *et al.*, 1968b). However, in contrast, Rosenzweig and Bennett (1969) were unable to detect significant dimensional effects of 30 days of differential rearing on either rat or gerbil cerebrum.

This apparent contradiction was resolved when Walsh *et al.* (1971, 1973) found that whereas 80 days differential rearing elicited a highly significant 2.5% increase in length, the 30 day effect was considerably smaller (1.2%). A subsequent series of experiments have confirmed the cerebral length increase for differential rearing periods ranging from 18 to 530 days (Cummins *et al.*, 1973, 1977; Cummins and Livesay, 1979; Kuenzle & Knusel, 1974). The effect appears to be more marked in animals brain damaged in infancy and subsequently exposed to differential environments (Will *et al.*, 1977).

Cerebral width effects have not been detected directly but the product of length and width has

proved the most powerful EC-IC discriminant. Perhaps environment exerts a similar though smaller effect on width as on length but this is too small to be detected by itself and is only made apparent when a product of the two measures is obtained.

In their original observation of behaviorally induced changes in length, Altman *et al.* drew attention to the presence of a mitotically active subependymal layer in the anterior forebrain of adult rats. Cells from this layer migrate to the anterior cortex and basal ganglia. Walsh *et al.* (1973) therefore reasoned that if modification of cell division in this layer underlay dimensional changes then they would be localized to the anterior regions of the cerebrum. This was found to be the case (Walsh *et al.*, 1973) and it raises the interesting question of the relationship of the cerebral length changes to those of cortical weight and depth. Since the length effect is limited to the anterior regions and cortical weight effects are more marked posteriorly they may well be largely independent of one another. This would be consistent with the finding that the weight and length effects appear to follow different temporal patterns (Walsh *et al.*, 1971; Cummins & Livesay, 1979). Whether the magnitude of the increase in length following complexity is a function of the unstimulated growth rate is unknown but it would certainly be fascinating to determine the effects of additional stimulation in the first 20 days of life while growth is rapid and the skull sutures remain open.

Cortical Depth

Following the repeated finding of an increase in cortical weight the logical question was whether an underlying morphological substrate for this change could be found. Microscopy provided an affirmative answer with the finding that changes in cortical depth paralleled those of weight (Diamond *et al.*, 1964, 1966, 1967, 1972, 1976; Diamond, 1967, 1976; Walsh *et al.*, 1972; Szeligo & Leblond, 1977). Following differential rearing from weaning the cortex is deeper in EC than IC animals. In general, the effect is greater in the medial than the lateral cortex with the former being more susceptible to increases and the latter to decreases in environmental complexity. It is maximal in the medial occipital cortex (around 7%) and minimal in the motor and somatosensory regions (Rosenzweig *et al.*, 1971). However, in the medial portion of the frontal cortex an EC-IC difference of 5.5% was

noted within an area 1.5 to 3.5 mm from the midline, being maximal medially (8.5%) (Walsh *et al.*, 1972). Effects in the somatosensory and frontal areas appear significantly larger at 30 than 80 days (Diamond *et al.*, 1972).

In summary then there is now considerable evidence that the depth of the rat cortex is modified by complexity-isolation in a regionally specific manner but as yet the functional significance of this patterning remains unknown. This cortical increase is clearly significant but as yet is not sufficient to fulfill Diamond's (1976) long held dream of causing the rat cortex to form its first convolution. In general the correlations obtained between weight and depth measures have been good (Diamond *et al.*, 1972). The advantages of depth, as opposed to weight, measures are that the former permit a much greater degree of spatial localization of changes, and also preserve brain for subsequent histological studies.

Hippocampal Thickness

In view of its function in memory formation it would seem logical to anticipate hippocampal involvement. To date however, results have been largely negative at least with regard to size and thickness, perhaps in part due to the marked individual variability which this organ displays. Walsh *et al.* (1969) reported that in the medial region underlying the occipital cortex the EC hippocampus was 5.7% thicker than that of the IC after 90 days. However, other studies have failed to detect significant effects on either thickness or weight at any of several different ages and durations of differential rearing (Diamond, 1976; Rosenzweig & Bennett, 1978; Cummins & Walsh, 1978; Jones & Smith, 1980; Walsh, 1980).

On the other hand, findings of changes in neuroglia, neuron nuclear, and dendritic morphology (Walsh *et al.*, 1969; Fiala *et al.*, 1978; Jones & Smith, 1980; Walsh & Cummins, 1979), clearly indicate significant hippocampal plasticity in response to the environment. At the present time it must therefore be concluded that if complexity affects hippocampal depth the effect is small and obscured by wide individual differences.

Corpus Callosum

As a consequence of the increased cortical activity following complexity one might anticipate a greater transfer of information between hemispheres and

consequent functional-anatomical-biochemical modification of the major transfer organ, the corpus callosum. And indeed Rosenzweig (Note 6) has demonstrated augmented transcallosal electrical conductivity and Altman and Das (1964) have indicated an increased number of autoradiographically labelled cells in the callosum from complex animals. More recently they have interpreted this finding as an indication of a greater production by the ventricular subependymal layers of new neurons and glia which subsequently migrate via the corpus callosum and other pathways to their definitive cerebral positions (Altman, 1970a). Callosal size may also be altered since Walsh *et al.* (1972) found that in the frontal, but not occipital, region the lateral, but not medial, callosum was 13.6% ($p < 0.01$) thicker following complexity. These findings will all require replication before being accepted, but they do suggest that the corpus callosum and interhemispheric conduction and integration may be adaptively modified by environment.

Diencephalic Dimensions

As previously noted it has been repeatedly found that while the weight of the cortex increases following exposure to environmental complexity, that of the subcortex (Rosenzweig *et al.*, 1971) decreases, though to a lesser extent. The microscopic substrate of increased cortical weight was found to be an increased depth and it therefore seems logical to anticipate that microscopy might detect a reduction, albeit small, in the size of the diencephalon. Diamond *et al.* (1966) were unable to detect such an effect underlying the occipital cortex while Das and Altman (1966), in the study cited previously, reported an increase in cross sectional area of 7%. However, this finding must be treated with great caution since the number of subjects was small, it is not clear that measurements were obtained blind, significance levels were not cited and the trend was opposite to that anticipated from weight.

Walsh *et al.* (1972) measured both breadth and depth of the diencephalon underlying the frontal cortex and found them to be reduced in EC by 3.8% and 3.3% respectively, although only the former reached significant levels. These findings are in the predicted direction and were obtained blind with larger numbers of subjects (15 littermate pairs) than other studies. They therefore suggest the possibility of subcortical size changes analogous

to the depth changes identified in the cortex. Further studies will be necessary to confirm them and determine whether a degree of subcortical regional specificity exists. The absence of any significant degree of localization for weight (Rosenzweig *et al.*, 1962) suggests that dimensional specificity, if it can be identified, will be small.

Colliculi and Cerebellum

Possible candidates for the detection of such changes might include the colliculi in view of their observed weight changes (Krech *et al.*, 1963) and the lateral geniculate because of the alternation in neuron nucleus: neuropil ratio (Malkasian & Diamond, 1971). On theoretical grounds one might also expect cerebellar components since studies in related fields point to the necessity of active self-guided exploration and kinesthetic feedback (reafference) in addition to passive examination of the environment from a distance (exafference) in order for learning to occur (Dru *et al.*, 1975). Furthermore, active interaction with the toys appears necessary for the full cerebral EC effect (Ferchmin *et al.*, 1975) and since the cerebellum is the major kinesthetic feedback and integrative organ environmental effects may well be detectable. Prescott (1970) in particular has laid theoretical emphasis on the role of kinesthesia and the cerebellum in development of the CNS. However, as a complex heterogeneous organ it may well be that there is a degree of response localization such that while effects for the whole organ are extremely small (Rosenzweig *et al.*, 1971) various components may be particularly affected (Floeter & Greenough, 1978). This is analogous to the small effect on whole brain weight.

NEUROHISTOLOGY

Having identified dimensional changes within the cortex as a result of environmental complexity and isolation the next logical question becomes "what constituents contribute to this change?" This question has been more methodically and carefully analyzed than in related research areas such as early handling, dark rearing, etc., and a variety of histological changes ranging through cell numbers, types, and sizes down to synaptic numbers and sizes have been identified. Effects on the medial occipital cortex are summarized in Table I.

TABLE I

Summary of anatomical changes measured in the medial occipital cortex. Except where indicated, data are from studies by Diamond and her coworkers and the table is partially based on Diamond (1976).

Measurement and condition	N (pairs)	Experimental duration in days	Percentage difference	p
I Cortical depth difference between enriched and impoverished rats				
A. Pre-weaned Long-Evans males	7	6-14	2	N.S.
	7	6-19	7	0.01
	19	6-26	9	0.01
B. Wistar males (Walsh <i>et al.</i> , 1969)	8	25-115	4.9	0.05
C. Postweaned S ₁ males	24	60-61	0.7	N.S.
	20	26-30	4	0.001
	24	60-64	3	0.01
	22	25-32	4	0.001
	23	25-40	7	0.001
	30	25-55	7	0.001
	50	60-90	7	0.001
	22	25-105	8	0.001
	18	105-185	5	0.05
	22	25-185	3	0.05
D. Castrated Long-Evans male neonates	11	60-90	6	0.05
E. Castrated Long-Evans males at 30 days of age	12	60-90	7	0.001
F. Nonpregnant Long-Evans females	23	60-116	4	0.001
G. Postpartum Long-Evans females	21	60-116	3	N.S.
H. Newborns from Long-Evans enriched and impoverished	30	95-116	0	N.S.
II Cortical depth difference between enriched and standard colony postweaned S ₁ males	20	26-30	0.5	N.S.
	24	60-61	4	0.001
	23	25-40	3	0.05
	22	25-55	3	0.05
	21	60-90	5	0.001
III Cortical depth differences between standard colony and impoverished S ₁ males	20	26-30	3	0.01
	24	60-64	1	N.S.
	23	25-40	5	0.001
	22	25-55	2	0.05
	21	60-90	4	0.05
IV Difference in glial number between enriched and impoverished S ₁ males	17	25-105	14	0.01
EC-IC glial differences (IC and SC did not differ significantly) (Szeligo & Leblond, 1977)				
Oligodendroglia	14	20-50	27	0.01
	10	20-100	33	0.01
Astroglia	14	20-50		N.S.
	10	20-100	13	0.01
Oligodendroglia	8	20-115	-3.6	N.S.
Astroglia	8	20-115	6.2	N.S.
V Difference in neuronal area between enriched and impoverished S ₁ males	12	60-90	4	0.05
	20	25-105	12	0.001
VI Difference in neuronal nuclear area between enriched and impoverished S ₁ males	20	25-55	2	N.S.
	19	60-90	2	N.S.
	20	25-105	12	0.001
	18	105-185	-1	N.S.
VII Difference in postsynaptic length between Enriched and standard colony S ₁ males	13	25-55	1	N.S.
Standard colony and impoverished S ₁ males	13	25-55	6	0.05
Enriched and impoverished S ₁ males	13	25-55	8	0.01

Cortical Differential Cell Counts

Neuron density In 80 day experiments cell counts have revealed a decrease in neuron numbers per unit cortical volume in complex as compared with isolated animals. Diamond *et al.* (1964) found that in the occipital cortex the reduction per microscopic field amounted to 17%, was maximal in layers II and III, not apparent in layer I and reversed in layers IVa and Vb. In the somatosensory cortex the reduction in density was less marked (7%) and occurred in layers II, IV and V, while layers III and VI were less consistent and occasionally showed reversal. A similar pattern was noted by Cummins and Walsh (Note 4) who found a 13.5% effect which was not evident in layer I, was maximal in layers II, III, and IVa, and was reversed in layers IVc and Va.

The absence of depth changes in layer I has been reported following direct measurement in several areas (Diamond *et al.*, 1964, 1966; Walsh *et al.*, 1969, 1972) and is consistent with the absence of neuron density changes in this layer. Unfortunately, layers other than the first are not clearly demarcated in the rodent cortex and hence are not susceptible to direct measurement.

Diamond *et al.* (1964) assumed that the total number of neurons was fixed and that reduction in density was therefore indicative of dilution and could be used as an index of cortical volume expansion. However, subsequent experiments have not supported this index (Diamond *et al.*, 1966). Furthermore, Cummins and Livesay (1979) have proposed that isolation may exacerbate the reduction in cortical neuron numbers that occurs after weaning. Therefore at the present time care must be taken in interpreting the meaning of both neuron density and neuron:glia ratio measures.

Since the maximum effect occurs in layers II and III, it is noteworthy that EC-IC differences in nuclear and perikaryon diameters were also maximal in these same layers (Diamond, 1967). In the light of these findings it is perhaps of significance that layer II increases markedly in width and cell density as the phylogenetic scale is ascended. The results may support Altman's (1967) hypothesis that the neurons of this layer represent the plastic regulatory components of the CNS. The significance of, and the reasons for, the absence of response in layer I and the apparent decrease in thickness of parts of layer IV and V of the EC cortex are unknown. Presumably causes

are to be sought in the differential composition and afferent supplies of these layers.

Neuroglia Diamond *et al.* (1964) found the density of neuroglia per microscopic field to be less in EC than in IC animals by 6.7% and 5.9% on the visual and somatosensory regions respectively (Diamond *et al.*, 1964). However, since the glia:neuron ratio was increased in the EC animals and since the total number of neurons was assumed to be fixed this was thought to indicate an increase in the total number of EC neuroglia. In the individual cortical layers the differences in glial density were so small that no trend could be observed.

In an effort to determine the glial subtypes responsible for the increase, cells were classified by nuclear morphology as astroglia, oligodendroglia, and intermediates (cells which exhibited characteristics of both astroglia and oligodendroglia) (Diamond *et al.*, 1966). The density of glia per microscopic field was 14% greater in the EC than in the IC occipital cortex and it was found that greater numbers of oligodendroglia and intermediates were responsible for this difference. These cell types showed greater densities in the EC brains of 20% and 36% respectively. Examination of astrocytes gave conflicting results since in the first experiment of this paper the EC rats had significantly more (27%, $p < 0.01$), but in a replication there was a slight reversal (-7%, NS). Combining the results of both experiments gave a greater density of astroglia (4.8%) but this finding failed to reach significance.

Eleven years later Szeligo & Leblond (1977) confirmed these trends by finding that 30 and 80 days of enrichment increased oligodendroglial densities by 27% and 33% respectively. The major effect seemed to have occurred by 30 days, but for astrocytes an increase was detectable only in the 80 day subjects and amounted to 13%. Isolated and social control (3 animals per standard cage) animals did not differ significantly and in no case were effects noted in the corpus callosum. Interestingly, it was found that handling neonates for 15 minutes daily for the first 10 days of life effected a 12% increase in astrocytes but not in oligodendroglia. This pattern of glial response differs from the EC-IC one and suggests either an age or stimulus specific response. It is interesting in light of the fact that astroglia have been found to proliferate earlier in life, during the preweaning phase, than oligodendroglia which tend to have a more marked postweaning proliferation.

A similar study by Cummins & Walsh (Note 4) was only partially successful. Similarly to Diamond *et al.*, the glia : neuron ratio was greater by 8% after 90 days of differential rearing. This was entirely due to astroglia such that the astroglia : neuron ratio was greater by 9%, though this difference was nonsignificant. The density of oligodendroglia was 3.6% less in EC than in IC cortex though once again this trend was nonsignificant and the number of intermediates counted was too small to allow meaningful interpretation. Likewise, no changes were observed in microglia, which are thought to represent blood derived phagocytic cells and hence to be distinct from other glia (macroglia). For this study then it can only be said that despite a suggestive trend towards a greater frequency of astroglia no significant differences in either macroglia or microglia were detected, and this in spite of very large glial effects in the underlying hippocampus (Walsh *et al.*, 1969). The only other report on cortical histology following complexity-deprivation is that of Altman and Das (1964) who reported an increase in the number of H^3 thymidine labelled cells in the corpus callosum and cortical radiation adjacent to the callosum of EC brains. These cells were initially assumed to be glia but as previously mentioned Altman (Note 1) now feels that they represent postnatally derived undifferentiated precursors of both neurons and glia.

In summary then, a complex environment appears to increase cortical glial density relative to both social and isolated animals. This increase is greatest for oligodendroglia which appear to exhibit maximal effects by about 30 days. Astroglia, on the other hand, display a greater 80 than 30 day effect. Astroglia also appear to be modified by neonatal handling. There are conflicting data on whether or not these effects may also be detectable in the corpus callosum. Although it has been implicitly assumed that the greater EC glia density represents the result of proliferation, the recent finding by Diamond (1976) that rat cortical glia numbers decrease with aging between 40 and 108 days of age, suggests that the EC effect might possibly represent, at least in part, a sparing or retention effect.

Hippocampal Differential Cell Counts

To date the only report of differential cell counts in the hippocampus is that of Walsh *et al.* (1969) who examined the medial hippocampus underlying the

occipital cortex of rats differentially reared for 90 days. The medial hippocampus is composed of two embryologically distinct components, the hippocampus proper and the dentate gyrus. Within both structures neuronal nuclei are aligned into compact and clearly demarcated layers which alternate with layers of neuropil, and it is within these neuropil layers that the counts were performed.

The number of neuronal nuclei in the neuropil is very small and as might be expected from this fact no significant EC-IC differences were detected (Table II). For neuroglia, however, the effects were highly significant for all glial subtypes except the microglia. For astrocytes the effect amounted to a 9.8% decrease in density in the complexity animals as compared with isolates.

Since, in this experiment, the EC hippocampus was of greater size than the IC, the lesser density of astroglia in the EC hippocampus was originally interpreted as a dilution effect due to an increase of internuclear material rather than a reduction in absolute numbers. Such an increase in internuclear material is suggested by the similar trend in occipital cortex (Diamond *et al.*, 1964, 1966) and the lower density of hippocampal blood vessels which will be discussed shortly. However, further analysis revealed that by far the major component of this difference lay in the Stratum multiformis layer of the dentate gyrus where other glial and neuronal changes were most evident (Cummins & Walsh, 1978). It may well be therefore that astroglial numbers are indeed altered within this part of the dentate gyrus.

The EC-IC differences in the number of oligodendroglia and "intermediates" per microscopic field reached 38.7% and 261.6% respectively (Table II). In spite of the low number of "intermediates" closer examination revealed that the EC induced changes in these two glial types was localized to positions which lay within the dentate gyrus rather than the hippocampus proper. Furthermore, there would appear to be a degree of localization even within the dentate gyrus, since EC-IC differences were maximal for both cell types within the so-called Stratum multiforme. These findings are interesting in light of reports that a proportion of the neurons of the dentate gyrus are formed postnatally (Altman *et al.*, 1968a, b; Shlessinger *et al.*, 1974). If, as these authors have hypothesized, the microneurons so formed represent plastic regulatory CNS components, then the glial changes noted in this study may be

TABLE II
Hippocampal differential cell count. Highly significant treatment effects were evident for astroglia, oligodendroglia and intermediates, but not for neurons or total macroglia. Based on Walsh *et al.*, 1969

Cell type	Mean number per animal		Percent difference	<i>p</i>
	EC	IC		
Neuron	9.52	9.46	0.6	N.S.
Astroglia	116.6	129.4	-9.8	<0.02
Oligodendroglia	29.5	21.26	38.7	<0.001
Intermediates	7.74	2.14	261.6	<0.0001
Total Macroglia	153.4	152.8	0.4	N.S.

especially associated with them. Since oligodendroglia are responsible for myelin formation (Maturana, 1960; Peters, 1960) it would seem possible that heightened axon development occurs in EC animals. It is impossible to determine whether these proliferating glia are perineuronal or interfascicular without the use of serial sections. That glia in the satellite position can proliferate following increased neuronal function, specifically motor activity, has been reported by Kuhlenkamp (1952). An attempt by Diamond *et al.* (1966) to answer this question by the use of serial sections of the cortex was unsuccessful.

The function of the glial cells with characteristics of both astroglia and oligodendroglia, and even their true identity, i.e., whether they are true intermediates in the process of transition from one glial type to another, is quite unknown. The question is a difficult one since the very existence of true intermediates is still debated, although work by Moliner (1958), and electron microscopic and autoradiographic evidence (Smart & Leblond, 1961) supports the concept of transitional glia. Brizee & Jacobs (1959) found that spongioblasts continue to divide into adult life and serve as a reservoir for oligodendrocytes so that possibly the EC conditions have stimulated precursors to enter the early stages of development and to differentiate into oligodendroglia.

The mechanisms by which an increase in neural activity leads to glial proliferation is unknown. One possible candidate involves the fact that potassium efflux occurs from discharging neurons

and that glial cell membrane potential and cellular metabolism are highly sensitive to extracellular potassium levels (Orkand *et al.*, 1966). We might hypothesize that the sequence of events and mechanisms mediating glial proliferation following environmental sensory stimulation in general, and complexity in particular, may be one of heightened neural activity modulating the membrane potential of glia and undifferentiated precursor cells, e.g., spongioblasts. These changes would in turn elicit alterations in metabolism via genetic derepression and possibly other mechanisms such as activation of RNA polymerase. The derepression would also induce mitosis. One might predict from this that glial RNA, protein, and size would be augmented following sensory input. Such findings have been reported for glial RNA following experiments involving learning (Hyden, 1967) and might also be predicted for complexity. The functions which might be subserved by an increase in glia are numerous and include support for newly formed fibers, nutrition, regulation of electrolytes, and postulated functions in memory formation (Diamond *et al.*, 1964).

In the above discussion it has been implicitly assumed that the "intermediates" do represent true glia but an alternative view has been suggested by Altman (1970): namely that they may be new or only partially differentiated neurons, at least in the dentate gyrus since it is here that Altman and his co-workers have identified some of the strongest signs of postnatal neuron formation. Such an interpretation would be pleasing from the point of

view of heightened neural activity eliciting the formation of additional neurons to process the extra input.

From the information available it seems impossible to determine which of these interpretations is correct, but one approach to the question might employ the injection of radioactively labelled thymidine at various intervals before sacrificing differentially reared animals. Newly formed cells would be radioactively tagged and their migration after various periods could be followed. If intermediates moved to the nuclear layers, particularly to the lateral aspect of the Stratum granularis as postnatally derived neurons are reported to do, then their neural identity could be assumed, but if they remained in the neuropil then they would more likely be neuroglia.

Neuronal Morphology

Perikaryon and nuclear size Neuronal size reflects the level of cellular metabolism and as such reflects the intensity, duration, and frequency of stimulation (Geinismann, 1971). It therefore seems reasonable to expect variations in sensory input such as complexity-deprivation to influence neuronal size, especially in view of the evidence for altered metabolism. Diamond (1967) measured neuronal perikaryon and nuclear size in the occipital cortex and found significant differential rearing effects, both preweaning (Malkasian & Diamond, 1971), and 80 days postweaning. The effect was greatest in the upper one third of the 80 day cortex where the mean EC-IC difference in perikaryon diameter was 16.7% and that of nuclei was 16.2%. This finding correlates well with neuron count data which indicate that the greatest cortical expansion occurs in the upper layers. Effects were less marked in the lower one-third (11.6% and 10.0%) and least in the middle one-third (7.2% and 7.6%). Thus, the main increase in both amount of internuclear material (Diamond *et al.*, 1964) and cell size was in the outer one-third of the cortex. There was also a positive correlation between nuclear size and cortical depth. However, when differential rearing was commenced later at 105 days of age, or when the duration of differential rearing was reduced to only 30 days, there were no significant effects on nuclear size. This was true whether the EC-IC program was commenced at either weaning or 60 days of age. However perikaryon dimensions displayed detectable differences (4.5%) with only 30 days treatment. This

confirms previous findings that nuclear size may not always correlate with that of the perikaryon (Zetterberg, 1966; Diamond, 1976; Diamond *et al.*, 1975).

In an interesting extension to primates Floeter and Greenough (1978) reared monkeys in either complex colony, social, or isolate environments for six months and measured neuron soma size in the cerebellum. Purkinje cell soma diameters in the nodulus and uvula were significantly greater in the colony than in either social or isolate animals.

Another form of environmental stimulation which might be labelled as complexity was used by Schapiro and Vukovich (1970). They subjected neonatal rats to a thrice daily 20 minute combination of tactile, thermal, auditory, and visual stimuli from day one to eight and examined pyramidal neurons in the occipital cortex after rapid Golgi staining. No effect on perikaryonal size could be detected even though dendritic spine numbers were increased. However, only a small number of neurons in five pairs of animals were examined and this procedure would probably be insufficient to detect differences of the magnitude reported by Diamond (1967). Also the reliability of the rapid Golgi technique for cell size measurements is unknown, so it is difficult to be certain how to interpret this report.

The only other information on neuronal size comes from Walsh and Cummins (1979). They attempted to follow up on the finding of hippocampal glial changes by measuring neuron nuclear size in the hippocampus and dentate gyrus after 80 days rearing. Both maximum and minimum nuclear diameters were measured and the ratio of the two computed as well as estimates of nuclear area and volume. In addition to the mean value in each animal for each of these parameters, their variability within individual animals was determined by the coefficient of variation (standard deviation divided by the mean). Interestingly enough, while no overall differences in size could be detected, environmental effects on variability were evident for all parameters. The effect was such that the coefficient of variation was significantly greater in IC than EC animals. However this effect was only evident in the Stratum granularis of the dentate gyrus. This is the same layer in which postnatally formed neurons have been reported (Altman, 1970) and lies adjacent to the Stratum multiformis where glial changes are most marked. The effect on ratio of diameters suggests more than a simple overall change in size but an

actual effect on nuclear shape resulting in increased pleomorphism (variability in shape). One interpretation might be that the greater variation in isolates reflects a more heterogeneous cell population possibly due to a greater formation of new neurons.

Alternatively it might be that newly formed neurons represent a more homogeneous, less differentiated picture than older ones. In this case, the results would reflect greater numbers of new neurons in the EC brains.

Another explanation of a more heterogeneous IC neuron population in this layer might be that the lesser stimulation and hence neuronal activity is such as to delay differentiation and maturation of newly arrived cells. It may be that the lack of stimulation stunts maturation of some neurons in this layer such that they do not approach their genetically determined ceiling levels. This might occur as a primary response of neurons to low levels of stimulation. Alternatively, it might reflect a greater variability in input to individual cells perhaps due to the formation of fewer well functioning pathways such that neurons involved in these pathways are highly stimulated while those not so innervated are hypoplastic. The difficulties with these last explanations are that they do not account for the effect on the Stratum granularis with no effect in the other layers unless neural activity follows a similar regional pattern. This is suggested by the proximity of the glial changes. Here again we are up against the problem of our current ignorance of the precise patterns of neural activity which follow rearing under complex and deprived environments. Without this knowledge it is extremely difficult to be certain of the interpretation of any regional and temporal patterning of anatomical and biochemical changes. This may even be particularly important for neuron size since it has already been shown that the duration, intensity and character of stimulation determine the direction, extent and general pattern of volume changes (Geinismann, 1971). In any event, this study is interesting in raising the possibility that the environment may be capable of modifying the process of postnatal neuronogenesis. To date there have been surprisingly few studies of neural variability and the only other report on coefficients of variation comes from Diamond *et al.* (1975) who did not observe any significant effects on cortical neuron nuclear size.

In any event, in view of the large amount of work involved in simply performing the measure-

ments of neuron size it would seem a worthwhile investment to add the relatively simple calculations to determine variability as well as mean size in future experiments.

Dendritic Morphology

"One might suppose that cerebral exercise, since it cannot produce new cells, carries further than usual, protoplasmic expansions and neural collaterals, forcing the establishment of new and more extended intercortical connections" (Cajal, 1895).

Over 70 years were to pass before the experimental validation of Cajal's hypothesis. In their original report of increased cortical thickness, Diamond *et al.* (1964) suggested increased dendritic ramification as one of the factors contributing to the greater EC cortical bulk. They based this hypothesis on a report that postnatal cortical development is largely due to dendritic proliferation (Eayrs & Goodhead, 1959) and that there is a correlation between neuronal perikaryonal size and dendritic branching (Clendinnen & Eayrs, 1961).

The Dendritic Tree

The first report on the size of the dendritic tree was that of Holloway (1966) who measured the number of intersections that dendritic branches made with a series of concentric circles placed around a neuron. He reported that after 80 days the layer II EC occipital stellate neurones made more intersections with the peripheral circles than their IC counterparts. Volkmar & Greenough (1972) reared Long Evans hooded rats from weaning for 30 days under conditions of EC, IC, and pairs in IC cages. In the medial occipital cortex the number and order of dendritic branching of layer II, IV and V pyramidal cells were measured. In EC all cell types displayed a greater branching of distal branches of basal dendrites. As compared with isolates, paired subjects showed only small effects in the most distal branches whereas EC effects were quantitatively larger and detectable in the intermediate as well as distal branches.

Greenough *et al.* (1973) next asked whether these findings were unique to the occipital cortex or could also be detected elsewhere. To answer this question pyramidal cells in layers II and IV of frontal cortex and layer IV and V of temporal cortex were examined and three different types of

parameters obtained. First, the intersections between dendrites and each of a series of concentric circles at 20μ intervals from the cell body were counted (Sholl, 1956). This measure is sensitive to the combination of number, length, and location (with respect to the cell body) of dendritic branches but less sensitive to differences restricted to a small portion of the neuron. Second, the number of branches at each order away from the cell body (or in the case of pyramidal cell oblique dendrites, from the apical shaft) was recorded. A branch from the cell body or apical shaft was defined as order 1, both branches past the first bifurcation as order 2, and so forth. This analysis is more sensitive to the differences in higher order dendrites which the authors previously found in occipital cortex following differential rearing. Third, the projected length of dendrites of each order was recorded for the frontal cortex neurons.

No systematic pattern of environmental effects on dendrites could be detected in the frontal cortex. On the other hand there was clear-cut effect of the litter from which the subject had come. Similarly for temporal cortex, analyses of apical branching by order, then of whole neurons by the intersection of dendrites with rings, showed only occasional effects. However, environmental effects were clearly apparent in the higher order branching of basal dendrites. In layer 4 the complexity animals had significantly more basal branching than their isolated and socially housed litter mates, while in layer 5 both environmental complexity and isolated animals had more basal branches than social rats. As in frontal cortex there were significant differences among litters on all measures.

The effects in this study are much smaller than those found in occipital cortex but are qualitatively similar in that the environmental effects appear to be restricted to basal dendrites, as also are effects on spine density (Globus *et al.*, 1973). Since these effects are not seen in all neuronal populations it seems doubtful that the environmental effects on dendritic branching are mediated by systemic factors such as hormonal or nutritional differences. The specific factors involved await further elucidation.

This still leaves unanswered the question of whether length as well as branching is affected, but the work of Globus *et al.* (1973) suggested that environmental effects on dendritic length may be quite small. They measured the lateral width of occipital cortex pyramidal cells in S1 strain rats which had been differentially reared for 30 days

from weaning. The lateral width and length of a pyramidal cell are measures of the diameter and length of the cylinder whose surface is defined by the ends of the terminal, oblique and basal dendrites (Globus *et al.*, 1973). EC and IC width values differed by 1.4% but this was nonsignificant. However, Uylings *et al.* (1978) reported that complexity increased the length of higher order dendritic branches of layer II and III pyramidal neurons in the occipital cortex of rats differentially reared during adulthood.

Recent work by Greenough (Note 5) supports the idea that dendritic length is also involved. He feels that there may be some "tradeoff" between bifurcations and length from one experiment to another, and that the most stable measure may be total dendritic length. If this is so it will be interesting to know what factors determine the nature of this tradeoff.

Dendritic effects also occur in areas of the sub-cortex. In the hippocampus Fiala *et al.* (1978) reported wider dendritic fields and greater lower order branching in neurons from the molecular layer of the dentate gyrus. This finding was noted following four weeks differential rearing from weaning but not after 12 weeks beginning at 21 weeks of age. Since lower order branches first develop at about weaning they are probably most susceptible to modification at this time. This is in contrast to the cortex where effects are localized to higher order branches, presumably since lower-order ones have already developed by weaning (Volkmar & Greenough, 1972).

Effects have also recently been reported in specific cell populations and regions of the monkey cerebellum (Floeter & Greenough, 1978). Following six months' differential rearing, animals from complex colonies displayed more extensive purkinje cell spiny branchlets in the nodulus and paraflocculus, but not in the flocculus. Granule cell dendritic branching however, displayed no such effects. These findings, together with the measures of purkinje soma size described earlier, are of particular interest in that they extend data on the effects of complexity-deprivation to primates, and to the cerebellum, and within this organ demonstrate a degree of regional and cell type specificity.

Dendritic Spines

Globus *et al.* (1973) also performed counts of the number of spines per unit length of dendrite and

found significant effects. Complexity produced an increase in spine numbers which was most marked in the basal dendrites (9.7%), smaller though still significant in the oblique and terminal branches (3.6% and 3.1% respectively), and apparently absent in the midsection portion of the apical dendrite. This finding is similar to that of Schapiro & Vukovich (1970) who, as previously mentioned, subjected neonate rats to thrice daily multimodality stimulation for eight days from birth. Under these conditions all types of dendritic branches displayed increased spines with maximum effect (33%) in the oblique and basilar, an intermediate amount (28%) in the terminal, and least (10%) in the apical. These effects are considerably larger than those of Globus *et al.*, a fact which the latter attributed to the younger age of Schapiro and Vukovich's subjects and the stressful nature of some of the stimuli used, e.g., mechanical vibration and electrical shock. Independent support for this finding is available from studies of light deprivation and cortical electrical stimulation, both of which have been found to elicit a variety of dendritic responses (Valverde, 1971; Rutledge, 1976).

The patterning of effects among the various branches is interesting in view of some claims about their principal connections. Apical branches receive most of their afferents from the specific afferent radiation, which in the case of the visual cortex is from the lateral geniculate, and it is these spines which are most affected by dark rearing (Globus & Scheibel, 1966; 1967a; 1967b; Valverde, 1968). These specific afferents would probably be little affected by environmental complexity or deprivation since the total amount of light input is probably about the same in both conditions and it is probably the cortical integrative functions which are most called into play by EC as opposed to IC conditions. Thus, it is interesting that it is the apical branches which show least effect and the basal, which receive primarily intracortical connections which display most. The oblique branches, which were the most affected in the Schapiro & Vukovich (1970) study but very little affected in the Globus *et al.* study, receive most of their afferents from the interhemispheric corpus callosum fibers (Globus & Scheibel, 1967b). Perhaps interhemispheric connectivity is more susceptible to modification in the neonate animal. It should be noted that this model of discrete inputs from separate sources has been seriously questioned (Peters & Feldman, 1976) and so the above interpretations should be accepted with caution.

Studies of dendritic spines employing light deprivation and stimulation suggest that there may be three classes of spines which can be differentiated according to their responses to visual experience. The first develops independent of stimulation, the second is dependent on stimulation but this stimulation may occur at any time, and the third requires stimulation at a specific developmental period. This finding comes from Valverde (1971; Valverde & Ruiz-Marcos, 1969) who examined spines on the apical dendrites of pyramidal cells in the visual cortex of mice. The stimulation independent spines developed around the time of eye opening at about 12 to 15 days of age whereas the second class appeared later whenever the light deprived animals were exposed to light. The third group of spines developed only when the animals were light exposed at the time of eye opening, i.e., they were both age and experience dependent. This "critical period" class of spines correlates well with the behavioral finding that the normally light exposed animal may reach a level of perceptual sensitivity which the deprived animal cannot achieve. The second class of neurons (stimulation dependent) is particularly interesting from a perspective of memory formation since it suggests the existence of a population of neurons that is made functional by experience (Greenough, 1978). Conceivably similar neurons could be waiting to encode experience in other brain regions.

Effects of Formal Training on Dendrites

Following their discovery of the effects of social and enriched rearing on cortical dendrites when compared with animals reared in isolation, Greenough *et al.* (1979) attempted to determine whether formal training could elicit similar effects. They therefore trained adult rats in two different types of procedures, the first a two alternative visual discrimination task, and the second a Hebb-Williams maze. Neither procedure brought about the large increases in stellate neuron or in basilar dendritic branching which they had formerly detected in the environmental studies. Rather, they noted that oblique or terminal branches from outer regions of layers 4 and 5 pyramidal cell apical shafts were longer in animals who had received the extensive formal training. Thus, as compared to the environmental studies in young animals, the effects are more subtle and located at the outer extremities of the dendritic tree.

Mechanisms Mediating Changes in Dendritic Spines

Since it is now clear that environmental stimulation is capable of modifying both spine numbers and morphology, the obvious question arises as to the mechanisms involved. A possible clue is available from the work of Van Harreveld and Fifkova (1975) who noted swelling in spines immediately following stimulation. They examined hippocampal granular cells and compared spines on the same dendrites where some spines were known to have been stimulated and others not. Thirty seconds of stimulation resulted in an enlargement of the cross sectional area of the stimulated spines which was present two minutes after stimulation and had not noticeably decreased after 60. They postulated that swelling resulted in a decrease in the spine electrical length resistance and this seemed one possible mechanism to account for post-tetanic potentiation. If this swelling is stabilized by a more enduring process such as the laying down of structural proteins, then it might also provide a basis for long-term morphological and functional effects. Van Harreveld and Fifkova suggest that this post-tetanic swelling may be similar to the swelling which has been observed following spreading depression (Van Harreveld & Khatlab, 1967). The uptake of electrolytes and water which causes the swelling seen during spreading depression is thought to be due to a release of glutamate from the intracellular compartment into the extracellular space, and this suggests that a similar mechanism may possibly underlie post-tetanic potentiation and environmentally induced effects.

Summary of Dendritic Effects

Thus it is evident that Cajal's predictions have been borne out. Dendritic arborization and numbers of spines are increased by a complex environment in both stellate and pyramidal cells but the effect on length of individual branches is less certain. There is a specific patterning of such changes with branching occurring in the more peripheral portions of the tree and the changes in spine density being greatest in those branches subserving intracortical connections and least on those receiving specific sensory afferents. Whether, like spine growth, environmentally elicited bifurcation occurs only in specific types of dendritic branches is not known. Presumably the greater arborization, plus the increased density of spines reflect axonal arborization and greater synaptic numbers.

Synapses

After decades of speculation (e.g., Cajal, 1895; Hebb, 1949) on the possible effects of environmental enrichment on synaptic morphology the first experimental support was provided by Mollgaard *et al.* (1971). Following 30 days of EC-IC rearing layer III neuropil of the occipital cortex was subjected to electromicroscopy and the size and number of Gray's (1959) type I synapses were measured. Type I synapses are associated with dendritic spines and are identified microscopically by the presence of round vesicles in the presynaptic bouton and an asymmetrical cytoplasmic thickening of the postsynaptic membrane. Hence they are sometimes referred to as asymmetrical axo-dendritic synapses.

EC synaptic length was greater than in isolates but synapses were less numerous per unit volume of cortex. That the increase in length was not due simply to a loss of small synapses was demonstrated by the fact that the EC animals possessed more large synapses as well as fewer small ones than did their littermates. It was estimated that the net effect of these changes was increased total synaptic area in the EC group. In this experiment cortical depth was found to be increased by 4% and the EC-IC littermate differences in depth correlated highly with synaptic length differences ($r = 0.79$) but not with synaptic numbers. Replication studies in the Berkeley laboratories have resulted in similar though considerably smaller effects, with SC values lying close to complexity ones (Diamond *et al.*, 1975b; Diamond, 1976).

These findings have been extended to other occipital layers by West & Greenough (1972) who measured bouton diameter and length of the postsynaptic thickening of type I synapses. After 30 days of differential rearing the postsynaptic thickening was significantly longer in EC than IC cortical layers I and IV but not VI, while the diameter of the presynaptic bouton was not appreciably altered. Unfortunately, synaptic numbers were not counted so it is not known whether the inverse relationship between size and number holds in this case. It should be noted that the findings of reduced synaptic numbers per unit volume in the EC cortex stand in contrast to the report of increased dendritic fields and dendritic spines which would be expected to predict greater numbers of synapses. One possible explanation is that the findings reflect a dilution effect within an expanded EC cortex.

However Cummins (Note 3) working with mice, has recently replicated his finding of greater synaptic numbers in the EC cortex (Cummins & Walsh, 1976) and, in an excellent example of interlaboratory cross validation, an examination of his photographs by Diamond suggests that they are employing equivalent criteria for synaptic identification. The reason for this trend which is in the opposite direction to other reports, is not yet apparent. Clearly the EC-IC conditions modify synapses but the final story on exactly how, remains to be told. In any event it is apparent that the effect of stimulus complexity as opposed to deprivation is to modify synaptic size, at least as far as the postsynaptic thickening of axodendritic synapses is concerned, and to modify synaptic numbers. SC values appear to be close to those of EC. The net effect is to increase the synaptic interface between neurons and presumably therefore to augment connectivity. Whether other components of the synapse also enlarge to support this increase in activity and whether other types of synapses are similarly affected remains to be seen.

Greenough *et al.* (1978) have recently added a further synaptic parameter to those found responsive to the EC-IC conditions. They measured subsynaptic plate perforations which are gaps in the postsynaptic thickening. They reported a higher proportion of occipital cortex synapses displaying perforations in EC than IC animals. However, in this study the postsynaptic thickening did not display the expected EC-IC differences in length so that additional caution is required in interpreting this finding.

Mechanisms Mediating the Modification of Neuronal Interconnections

It seems clear that experience is capable of modifying the numbers and morphology of dendrites and synapses, and probably also axons. What the mechanisms which mediate these effects are is by no means so certain. Alterations in the amount of axonal and dendritic growth by modification of the appropriate metabolic process is not difficult to imagine, but the question of how this growth would be directed towards appropriate neurons is not so obvious. Various chemical affinity mechanisms have been suggested for neural development and are probably essential for inducing basic developmental patterns of brain organization, but whether such a specification could include new information gained from experience is less certain. Perhaps electrically

active dendrites might secrete a chemotropic substance which would attract growing axons. Moore (1976) has suggested that spine activity might cause axons to sprout extra terminals, which would then compete for nearby synaptic sites.

Synaptic modifications have been suggested to be of several types, including new synapse formation, changes in size and morphology, and preservation of pre-existing synapses (Rosenzweig *et al.*, 1972). The preservation hypothesis has recently been gaining support from a number of areas and several authors have suggested similar but not identical models (Rosenzweig *et al.*, 1972; Changeux & Danchin, 1977; Greenough, 1978) and the following discussion owes much to their thinking, particularly Greenough (1978). The hypothesis contends that the brain and individual neurons initially make many temporary or tentative synaptic contacts and that only a proportion of these actually survive. It is further supposed that survival is based on their functional utility which in some cases would be partially determined by experience. Presumably fewer synapses would be preserved in stimulus deprived than in enriched animals.

Support for this preservation hypothesis comes from time course studies of both neurons and synapses. For example, in the frog spinal cord it appears that about four times as many neurons involved in limb muscle innervation are produced as survive to adulthood. This survival appears to depend upon a neuron's successfully competing with others to innervate a muscle (Prestige, 1970), and Hughes (1968) has suggested that this process may allow the selection of the most appropriate neurons for an organized neuromuscular control system. Indeed, selective survival may be commonplace in neural development, since neuron death occurs in a variety of places in the developing central nervous system, including the cortex (Diamond, 1976) and Cummins & Livesay (1979) have suggested that this may play a role in EC-IC histological differences.

A similar pattern may occur for spines and synapses. For example, Ruiz-Marcos & Valverde (1969) report that the number of spines in certain areas of mouse cortex reaches a peak shortly after weaning and then begins to fall toward an adult level, and that this final level is lower still in dark reared mice. The same picture occurs in cat visual cortex where synaptic density peaks near the end of the fifth postnatal week (Cragg, 1975). This peak matches the period of maximal sensitivity to

monocular deprivation (Hubel & Wiesel, 1970). Westrum (1975) described multiple axon contacts on single dendritic sites in early development, but only one axon terminal appeared to survive to maturity. Competition for synaptic sites has also been proposed in other developing systems, especially the visual where it is the basis of much of the monocular deprivation effects (Hubel & Wiesel, 1970).

A Dynamic Adaptive Homeostatic Brain

It is probably simplistic to think of neuronal interconnections in adult brain as fixed and static. Probably a more adequate picture is provided by a dynamic model in which axonal, dendritic, and synaptic growth is in constant dynamic flux. The net result would approximate homeostasis, unless sensory input or other modifying factors resulted in a shift towards another homeostatic balance point.

A dynamic adaptive homeostatic model of brain is probably also appropriate in more general terms. For what the data reviewed in this paper show is that the brain is a plastic organ which responds in an exquisitely sensitive and adaptive manner to the demands placed on it by its sensory environment. Both brain and environment modify each other and constitute an interactive ecological system. This is consistent with the increasing recognition in some other areas of science of the necessity of adopting dynamic holistic models which recognize the interdependence of components within a system (Walsh, 1979, 1980) and suggests that continued experimental refinement may result in the emergence of increasing cross disciplinary parallels at this level.

"We have only just begun, with the real questions and answers still ahead" (Diamond, 1976, p. 239).

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