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Early visual experience, learning, and neurochemical plasticity in the rat and the chick†

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Rats reared in the dark to 50 days show morphological and biochemical changes in the visual pathway. Light exposure results in elevated incorporation into protein in visual cortex, lateral geniculate and retina. Much of the visual cortex elevation is in a rapidly labelling, rapidly transported neuronal particulate protein. There are concomitant changes in lysosomal and transmitter enzyme activity. In chicks exposed to an imprinting stimulus (a flashing light) there are elevations in RNA polymerase and RNA and protein incorporation in the anterior forebrain roof (a.f.r.) compared with controls. There are changes in adenyl cyclase, cAMP and AChE. Behavioural controls show that although there are general biochemical sequelae of light exposure, the elevation in RNA synthesis in the a.f.r. is not a result of motor, stress or sensory activity, but is correlated wih a measure of the learning of the stimulus characteristics. A model for neurochemical correlates of developmental plasticity, learning, and state-dependent transients is discussed.

Introduction

There are two properties of the nervous system implied in the concept of plasticity. First the system must be able to respond to short term and transient changes in the environment by a modulation of its output so as to adapt it to changing circumstances. However, these modulations must be brief alterations in what may, somewhat naively, be described as the 'normal ground state' of the system. Second, the nervous system must be able to respond to long term, repetitive or 'significant' alterations in the environment by long term or permanent changes in its ouput. Some of these long term changes are rather general sequelae of environmental modification or experience, especially during development, such as undernutrition, hormonal changes or enriched and deprived environments, which are known to affect cell number, dendritic branching and behaviour. These long-term environmental effects on brain and behaviour are examples of the plastic adaptation of the organism to experience, but must be distinguished from what behaviourists would in general regard as the very precise and predictable modification of behaviour in response to particular experiences which is described as learning. By learning is meant that set of processes whereby particular experiences have specific effects on behaviour, and the model adopted in this paper assumes that there is a continuity between those physiological, biochemical and morphological events in the brain which underlie experience in the broad sense and learning in the narrow sense (Rose 1970; Horn, Rose & Bateson 1973*b*; Rose, Hambley & Haywood 1975).

† The experiments and ideas discussed in this paper are the result of collaboration with P. P. G. Bateson, Subdepartment of Animal Behaviour, Madingley, Cambridge, G. Horn, Department of Anatomy, Bristol, and J. Hambley, J. Haywood, A. Jones-Lecointe, K. Richardson & A. K. Sinha of the Brain Research Group at the Open University.

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The experiments I wish to discuss are first designed to map some of the neurochemical correlates of experience in the broad sense; and secondly, to examine a particular experimental situation in which learning can be shown to occur, and to ask which features of that experience are particularly correlated with which neurochemical responses. In describing such experiments, I am aware that other neurobiologists sometimes question the value of biochemical studies in this area. They argue that it suffices if one can demonstrate physiological changes, for example in the response properties of defined neurons, or that unless the anatomical locus of any change is precisely defined, its biological meaning is very much reduced. However, long term plastic changes, as opposed to the short term transients referred to above, must demand the structural modification or reorganization of the connectivity of the nervous system. I cannot envisage any way in which such structural modification can occur without involving biochemical processes, to modify membranes, generate new synaptic connections, alter transmitter levels, or whatever. What is more, unless one has a 'point locus' memory model, one may expect such changes to be generalized over a relatively large number of cells, perhaps over a diffuse region. In this sense biochemical correlates of plasticity are perhaps analogous to the study of evoked potentials in physiology; they represent the sum of small changes in a rather large number of cells, rather than large changes in a few. Biochemical changes occurring in a given region may also act as signposts to physiologists and anatomists that the region merits their attention too.

This is not to go along with some molecular biologists who would apparently argue that the properties of the nervous system can be 'solved' exclusively at the molecular or cellular level, of course, but simply to insist that the biochemical changes which take place are one part of the hierarchical jigsaw we are trying to assemble. Nor is it to assume that *because* biochemical changes must in principle take place in response to experience, the present level of technology is *of necessity* adequate to detect them. Biochemists are always working with small differences in rates of production of molecules, in levels of metabolites and in enzyme activity, which are barely at the level of reliable detectability. As the anatomical sites of changes are narrowed down and the biochemical effects fractionated out, the changes are magnified, from differences of the order of 10–20 % in incorporation or enzyme activity to differences of several fold. But it must always be expected that the 'additional' biochemistry which the fixation of experience requires will be a relatively small increment over the cell's 'normal' metabolism, and the smaller the experience the smaller the biochemical change or number of cells concerned. This is the argument for working with strong functional stimuli or 'important' learning events in the first instance. And small changes are no less real or 'significant' to the organism merely because they are small.

But it must not be assumed that, because biochemical changes are found associated with a particular experience, then these changes must be the necessary, sufficient and exclusive correlates of this experience. Those changes that are detected may, unless the necessary controls to exclude such possibilities are performed, be artefacts – for instance an apparent elevation in protein synthetic rate may merely reflect enhanced uptake of radioactive precursor into the cell as a result of altered blood flow or changed levels of free pool amino acids, or alterations in the rate of degradation of proteins (Rose, Hambley & Haywood 1975; Haywood, Hambley & Rose 1976). Or the changes may lie on a biochemical sidepath to the 'real' correlate – for instance, a genuine underlying biochemical correlate may be a process requiring ATP, and the level of ATP may be rate-limiting for protein synthesis. Hence protein synthesis will be modified indirectly when plastic responses to experience and learning occur. Finally, those

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changes that we detect may be biochemical correlates not of learning at all, but of a feature of the organism's behaviour which is a precondition for learning – an alteration in sensory input or arousal level, for example (Bateson 1970, 1976). It is against this background that the experiments to which I now turn must be judged.

Visual deprivation and stimulation in the rat

In the first series of experiments, we have examined the biochemical sequelae of a strong, functional stimulus to the organism without, so far, having made any attempt to dissect out those features of the animal's undoubtedly complex response to the experience most directly associated with the observed biochemical changes. The experience is that of visual deprivation followed by light exposure in the rat, and the biochemical changes which will be described are those of the altered production of particular proteins and changed levels of activity of certain enzyme systems. It is germane to these experiments to note that dark-rearing and light-exposure have been reported to result in morphological changes in the cortex including modification of synaptic size and density (Cragg 1967) and number of synaptic vesicles (Vrenssen & De Groot 1974, 1975), as well as in dendritic branching and spine formation as measured by Golgi methods (Valverde 1967, 1971).

Pregnant female Wistar rats were placed in light-proof wooden boxes housed within an otherwise normal animal house environment. The male offspring were reared in the dark until 50 days of age, when a group were exposed to controlled general illumination in individual cages ('light', L) while the rest were placed in individual dark cages ('dark', D). After varying periods of exposure the L animals and their control Ds were injected intraperitoneally with [^{3}H]- or [^{14}C]lysine, used as a precursor for protein; sometime later (generally 1 h) the animals were killed and 'visual' and 'motor' cortex regions removed. The cortex and other control regions were either processed directly to measure enzymes or incorporation of radioactivity into total tissue protein as compared with 'pool' or 'free' intracellular radioactivity, or the tissue and proteins further fractionated as described below (technical details are in Rose 1967*a*; Richardson & Rose 1972).

The initial observations made, in 1967 and extended in 1972 (Rose 1967*a*; Richardson & Rose 1972), were that when incorporation of lysine into protein is compared in L and D animals, there is a transient elevation of incorporation into visual cortex protein between 1 and 3 h after the onset of exposure of the L animals. With a 1 h pulse of radioactivity, the elevation in incorporation is maximal, about 20 % above control levels, after 1 h of exposure to the light. However, within 3 h the incorporation is back to control levels, and if exposure is prolonged thereafter, then depending on the conditions of illumination, there is a depression of up to 20 % in incorporation in Ls compared with Ds which may last for up to 4 days. A similar transient elevation in incorporation, followed by a long lasting depression, occurs in the lateral geniculate and retina (where the effect is much larger than in the cortex). However, there is no elevation in the motor cortex, and there is no change in incorporation rate in the liver.

Thus we could detect an apparent transient elevation in protein synthetic rate along the visual pathway following the first exposure to light of dark reared rats. In the further study of the biochemical significance of this effect we have concentrated on the visual cortex at the height of the elevation, 1 h after onset of light exposure.

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Are specific proteins being synthesized?

From the biochemical point of view a key question is whether the apparent elevation in protein synthetic rate is real, or merely reflects changes in precursor pools, blood flow or turnover. There is evidence both from our laboratory (Rose 1972; Haywood, Hambley & Rose 1976) and others (Bondy, Lehman & Purdy 1974) that visual stimulation and deprivation does result in altered cerebral blood flow and intracellular amino acid pools. However, we argued that, if we could show that the elevation of precursor incorporation was not general to all proteins but was confined to only a limited number, it would be difficult (though not impossible) to account for this in terms of precursor effects, and a more probable interpretation would be in terms of an alteration in the synthetic or turnover rate of those particular proteins.

To approach this question, we therefore labelled the proteins of 1 h L and D animals as before and, following the labelling, fractionated the visual cortex proteins. The labelled proteins were first divided into soluble and insoluble fractions and each group was then further subdivided, using polyacrylamide gel electrophoresis (Richardson & Rose 1973). To simplify the fractionation procedure, as we were looking for small differences which might be masked by 'noise', we used a double labelling method which enabled us to separate the visual cortex proteins into 21 soluble and 20 insoluble fractions; of these, 2 soluble fractions showed reliable elevations of incorporation of up to 53 %; 4 insoluble fractions showed elevations of up to 35 % and 1 insoluble fraction showed a depression of 60 %. There were no changes in the overall protein patterns observed by a densitometric scanning of the gels from D and L animals, and although it must be remembered that this is a fairly crude fractionation of the several thousand protein species which must be present within the tissue, the general results are compatible with the statement that part of the cellular response to visual stimulation of hitherto dark-reared animals is an enhanced rate of synthesis of a limited number of protein fractions in the visual cortex.

Gel fractionation is a useful diagnostic technique, but it is difficult to further subfractionate the gel bands which may be of interest, partly because the gels are capable of receving only very limited quantities of material, and the effects we are studying are themselves small. It seemed preferable to try an alternative approach to purification of the fractions in bulk.

This has involved subcellular fractionation of the visual cortex. Tissue from 1 h L and D lysine-injected animals was homogenized either separately, or, in double labelling experiments, after pooling, and subjected to a standard subcellular fractionation protocol (Jones-Lecointe, Rose & Sinha 1976). In the primary subcellular fractions, a difference between L and D in the visual cortex appeared only in a ribosomal pellet which sediments through sucrose (240 %; $P < 0.01$). There was no difference, for example, in labelling in the synaptosome fraction. The elevated labelling of a ribosomal fraction which contains less than 3 % of the protein of the initial homogenate may reflect a change in the rate of production of ribosomes themselves, or represent label present in nascent protein bound to the ribosomes but due to be released into the cell cytoplasm. So far we have not distinguished between these possibilities, though we consider the second to be the more probable, especially as subfractionation of the crude nuclear fraction does not reveal any differential labelling between L and D, although this might have been expected if new ribosomal synthesis was occurring. On the other hand, chromatographic separation of the cytoplasmic proteins has shown elevated incorporation into at least three soluble fractions (A. Jones-Lecointe, unpublished).

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Are the changes neuronal or glial?

In cerebral cortex from normal animals, the rate of synthesis of protein is higher in neurons than in neuropil or glia, as has been known for some time on the basis of autoradiographic (Droz & Koenig 1970) and electron microscopic (Palay & Chan-Palay 1972) evidence. Recently we have directly confirmed this by measurement of the rate of incorporation of precursors into protein in neuronal cell bodies and glia (neuropil) isolated by a density gradient method (Rose 1967*b*, 1975), both *in vivo* and *in vitro*. Following 1 h of incorporation of [^{3}H]lysine, specific radioactivity of the neuronal cell bodies is about 1.3–1.6 fold higher than in neuropil. What would happen if we compared incorporation into neurones and neuropil from the visual cortex of 1 h L and D animals? Table 1 shows that compared with normal animals, incorporation into visual cortex neuronal protein is depressed in dark reared animals (incorporation ratio 0.67 compared with 1.45) but that after 1 h of light exposure neuronal but not neuropil incorporation increases once more (ratio 0.98; $P < 0.001$). This effect is region specific, and in the motor cortex of the dark-reared animals neuronal incorporation is at the normal control level. A fraction of visual cortex neuronal protein synthesis is thus suppressed in dark-rearing and is switched on when the animal is exposed to the light (Rose, Sinha & Broomhead 1973).

TABLE 1. INCORPORATION OF [^{3}H]LYSINE INTO PROTEIN IN NEURONS AND NEUROPIL IN NORMAL, DARK-REARED AND LIGHT-EXPOSED RATS

brain region	incorporation period (h)	neuronal/neuropil incorporation ratio in		
		normal	dark-reared	light-exposed
visual cortex	1	1.45 ± 0.18	0.67 ± 0.03	0.98 ± 0.09
	4	0.35 ± 0.04	0.75 ± 0.05	—
	24	0.44 ± 0.06	0.70 ± 0.05	—
motor cortex	1	1.58 ± 0.09	1.35 ± 0.13	1.45 ± 0.07
	4	—	—	—
	24	—	—	—

Incorporation ratio is disintegrations per minute per mg protein in neuronal fraction/disintegrations per minute per mg protein in neuropil fraction after intraperitoneal injection of 250 μCi [4,5-^{3}H]lysine in 1 ml 0.9% saline and after the incorporation period shown. Dissections were made and cells separated as described in the papers from which this table is compiled (Rose *et al.* 1973; Rose & Sinha 1974*b*). Data are given as mean ± s.e.m of n of between 5 and 25. Differences between N and D in visual cortex are significant ($P < 0.01$) at all times; differences between D and L and L and N at 1 h are significant at $P < 0.01$ and < 0.05 respectively.

We can tell more about the nature of this suppressed fraction of neuronal protein synthesis from a study of the kinetics of incorporation of precursor into protein in normal animals (Rose & Sinha 1974*a*). Although the neuronal/neuropil incorporation rate is 1.3–1.6 following an hour of labelling in normal animals, if the labelling period is prolonged, within 4 h there is a dramatic fall in the ratio to around 0.4–0.6. Thereafter it is stable for several days. We have interpreted this data as meaning that in the neurones of the 'normal' animal there is a rapidly synthesized protein fraction which, present in the cell body after short labelling periods, is transported out over a few hours. From other experiments, based on the use of inhibitors of axonal flow, we have concluded that the rapidly labelling neuronal protein is part of the axonally or dendritically transported neuronal glycoprotein material (Rose 1976; Rose & Sinha 1976).

We were struck by the similarity of the neuronal/neuropil incorporation ratio in normal

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animals found after 4 h, and hence after the export of the rapidly labelling component, and the ratio shown after 1 h in the dark-reared animals. Could this be because the exported component of neuronal protein synthesis is absent in visual cortex in dark-rearing, or is there a general suppression of neuronal protein synthesis under these circumstances? The test is easy; it is only necessary to extend the labelling period in dark-reared animals to 4 h and perform the neuronal/neuropil fractionation. If there is a general suppression of neuronal labelling, a ratio of about 0.6 at 1 h should be reduced to around 0.2 at 4 h and thereafter. If the inhibition is specific to the rapidly labelled and exported component, however, the neuronal/neuropil incorporation ratio in D animals, low at 1 h, should not be altered by increasing the labelling period to 4 h. Table 1 shows the results of this experiment, which are unequivocal. In the visual cortex of D animals the neuronal/neuropil incorporation rate is constant over 24 h following injection (Rose & Sinha 1974*b*).

The interpretation of this series of experiments, together with the other data referred to above, is that the synthesis of a rapidly labelling, particulate glycoprotein axonally transported neuronal fraction is inhibited in visual cortex during dark-rearing, but is switched on at the onset of light stimulation. We are at present investigating the biochemical nature and precise localization of this fraction further, and have also found evidence for both transient and more long term changes in enzyme activity of a number of enzyme systems during dark-rearing and following light exposure (Sinha & Rose 1976).

Imprinting in the chick

Whereas in the experiments which have so far been described we have been concerned with the neurochemical correlates of plasticity in the broad sense, in our studies of the chick we have been working with an experimental situation in which a specific form of learning occurs. Imprinting is that process (Bateson 1966) whereby the recently hatched chick quickly forms a social attachment to a conspicuous object as a result of being exposed to it, and it involves the first significant visual experience for the bird. The merits of imprinting, which may be regarded as a special case of learning (Bateson 1971) are not merely that it clearly represents a very important experience for the young bird, substantially influencing its subsequent behaviour, but that many aspects of the experience can be controlled and manipulated. The exposure and test situations enable a battery of behavioural measures to be made, including approach latency, running activity, distress and contentment calls, as well as the direct measure of preference for the familiar object – that is, the degree of learning.

Our strategy in the chick studies, which involve an ongoing collaboration with Dr Bateson in Cambridge and Professor Horn in Bristol, has been first to define a relatively large anatomical region in which biochemical changes occur during learning; secondly to attempt to show that such changes are specific correlates of the learning; and only third (the stage we are now entering) to focus down more precisely on the exact sequence of biochemical events and their more precise anatomical localization.

The chicks are hatched and maintained in a communal brooder in the dark until a little before the optimal period for imprinting, some 18–24 h post-hatch. Chicks are then either removed to individual dark pens or wheels or are placed in pens or running wheels facing the imprinting stimulus, a coloured flashing light. After appropriate periods of exposure and rest they are tested for their preference for the familiar object (Bateson & Wainwright 1972; Bateson

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& Jaeckel 1974). Either before, during or after training or testing, radioactive precursors may be given by intracardiac injection. Samples are taken from several different brain regions: the mid-brain, containing the optic tectum and most of the thalamic nuclei, the base of the forebrain, containing predominantly neo-, ecto- and palaeostriatum, the posterior forebrain roof, containing hippocampus, and the anterior forebrain roof, containing hyperstriatum. The samples are then processed for radioactive measurement or enzyme assay as in the rat experiments.

TABLE 2. CHANGES IN UPTAKE AND INCORPORATION RATES OF PRECURSORS IN CHICK BRAIN DURING AND AFTER EXPOSURE TO AN IMPRINTING STIMULUS

system	stimulus onset (offset) to killing (min)	incorporation time (min)	brain region	100 I/D
[^{3}H]uracil into RNA	76	150	roof	120
	150	150	all regions	~ 125
[^{3}H]lysine into protein	150	90	roof	117
[^{14}C]lysine into protein	80 (20)	20	anterior roof	125
[^{14}C]lysine into pool	80 (20)	20	all regions	~ 121
[^{14}C]2-amino-isobutyrate into pool	80 (20)	20	all regions	~ 122

Incorporation, as disintegrations per minute per mg protein, either in acid insoluble (RNA and protein) or acid-soluble (pool) fractions from the studied brain regions from day old chicks, is expressed as that into (imprinted/dark controls) × 100. Only differences which are significant at $P < 0.05$ or better are shown; n is 15 or more for each condition. No significant differences occur at other times or regions.

In the ^{3}H precursor experiments incorporation took place during exposure; in the ^{14}C precursor experiments it took place immediately following exposure. [^{14}C]2-amino-isobutyrate is a non-metabolizable amino acid used as a marker for uptake (and hence probably blood flow) changes. Data are calculated from Bateson *et al.* (1972) and Haywood *et al.* (1976).

Precursor incorporation and enzyme changes

I shall not review all the experiments in the chick series here; the earlier results are summarized in Bateson, Horn & Rose (1972) and reviewed in Horn *et al.* (1973*b*). These experiments showed that there is increased incorporation of [^{3}H]uracil into RNA and [^{3}H]lysine into protein in the forebrain roof of chicks which have been exposed to the imprinting stimulus for 78 and 105 min respectively, but not in birds exposed either to diffuse overhead illumination or kept in darkness. Longer exposure results in RNA incorporation changes which are not region-specific. Birds exposed to the imprinting stimulus and then replaced in the dark continue to show elevated incorporation of [^{3}H]lysine into protein in the anterior forebrain roof, and a rather generalized elevation of uptake of precursor into all brain regions compared with dark maintained controls, for up to an hour (Haywood *et al.* 1976). These changes are preceded by an increased activity of the enzyme RNA polymerase in cell nuclei isolated from the forebrain roof of birds exposed to 30 min of imprinting stimulus, but not to shorter (15 min) or longer (45 min) periods of exposure (Haywood, Rose & Bateson 1970, 1975).

These observations are an advance over those with the rat in that they show a temporal sequence of changes following stimulus onset which are compatible with an activation of a transcription–translation sequence. The existence of a biochemical sequence of this nature, including a transient change in an enzyme activity (which cannot be explained away as a simple precursor artefact) is encouraging, although we have yet to demonstrate that the changes in incorporation of precursor into protein are limited to certain specific protein fractions as is the case for the rat first exposure. They also demonstrate that at the biochemical

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level, at least two effects, one rather general, affecting all brain regions, and one specific to the anterior forebrain roof, are occurring. This essentially represents the key conclusion to the first phase of our strategic approach. The precursor effects are summarized in table 2.

As both transient effects before RNA polymerase elevation and those involved in short term memory processes are likely to involve membrane and transmitter systems, we have made a temporal study of the cAMP-adenylcyclase and acetylcholine systems as possible candidates. Table 3 summarizes the effects we have observed. The shortest time effect we have found is a lowering of cAMP levels in the forebrain roof within 15 min of onset of stimulus, while effects on adenylcylase and choline-acetyltransferase occur within 1 h. There are apparent oscillations in acetylcholinesterase levels for up to 6 h after the end of the stimulus period in several brain regions. Neither the biochemical nor the behavioural significance of these changes is very clear, although the magnitude of some of the effects, especially in adeylcyclase, is large.

TABLE 3. CHANGES IN METABOLITE LEVELS AND ENZYME ACTIVITIES IN CHICK BRAIN AFTER EXPOSURE TO AN IMPRINTING STIMULUS

system	stimulus onset (offset) to killing (min)	brain region	100 I/D	reference
cAMP	15	roof	35	1
	15	mid-brain	160	1
RNA polymerase	30	roof	134	2
adenyl cyclase	30	mid-brain	78	3
	60	roof	150	3
choline acetyltransferase	60	mid-brain	110	4
acetylcholinesterase	120 (60)	roof	111	4
	420 (360)	roof	113	4
	420 (360)	base	112	4
	420 (360)	mid-brain	110	4
	780 (720)	mid-brain	87	4

Enzyme activities were determined in brain regions from day old chicks at varying times after exposure to imprinting stimulus. Maximum length of stimulus exposure, 60 min. Data is expressed as (activity in imprinted/dark control) $\times 100$, and only significant elevations or depressions are shown ($P < 0.05$ or better; n is 12 or more for each condition). Data are calculated from (1) Hambley *et al.* (1972); (2) Haywood *et al.* (1970); (3) Hambley & Rose (1973); (4) Haywood *et al.* (1975).

Behavioural specificity

So far, the results discussed for the chick are broadly compatible with those reported in the previous sections with the rat. While they help build up a picture of an organized biochemical response in the visual system to the onset of both general and more specific visual input, they do not tell us anything about the behavioural specificity of the effect. The responses, both in the forebrain roof and more generally, might have nothing to do with the learning of the imprinting stimulus *per se*, but may be sequelae of many other aspects of the bird's behaviour on exposure to the stimulus situation; motor or stress activity, sensory input, arousal or attention, or perhaps the indirect consequences of enhanced levels of circulating hormones. The advantage of the chick imprinting system, however, is that we can test and potentially eliminate these possibilities; the second phase of our strategy. Three behavioural controls, all using the single biochemical measure of uracil incorporation into RNA, will be described.

The first (Horn *et al.* 1973*a*) uses the fact of the complete decussation of the optic tract, which makes possible a 'split brain' preparation. In these experiments, the supraoptic commisures of

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12 chicks were divided shortly after hatching, and after recovery from the operation, one eye of each chick was covered with a patch. The animal was exposed to the stimulus for 1 h, then tested first with the trained eye and then with the untrained eye. No transfer of learning took place; there were no differences in pool between trained and untrained sides of the brain (ruling out assymetric blood flow); but incorporation into RNA in the forebrain roof (but no other region) in the trained side was significantly elevated by comparison with the control side. Hence it is unlikely that the changed incorporation results from general hormonal response to stress, non-visual sensory input or differences in motor activity.

However, trained and untrained halves of the brain also differ in the amount of visual input they receive. To test this as a possible cause of the biochemical changes the effects of duration of learning before precursor incorporation were examined (Bateson, Rose & Horn 1973). Birds were exposed to the stimulus for 20, 60, 120 or 240 min on the first day after hatching in the absence of precursor. On the second day, they were injected with [^{3}H]uracil and all exposed to the stimulus for 60 min. None of the groups differed from the others in activity during training during the second (60 min) exposure, but the incorporation into the anterior part of the forebrain roof was negatively correlated with duration of exposure on day 1. That is, those birds with the least exposure on day 1 – and therefore, we reason, the most to learn on day 2 – showed the highest incorporation in the anterior forebrain roof. This was not due to a 'carry-over' effect from the first day's treatment because the incorporation of precursor into birds kept in the dark on day 2 was identical irrespective of their treatment on day 1. This experiment would tend to rule out sensory stimulation *per se* as a trigger for the biochemical effects. In addition the results run counter to any 'vigilance' interpretation of the biochemical effect, as the birds' activity did not differ on day 2 between the groups exposed for varying periods on day 1. However, it might be argued that the onset of visual stimulation on day 1 had initiated a process of neuronal differentiation which, in the case of short-exposure birds, was not complete by day 2, and that this process was not necessarily related to memory or learning; this possibility is not ruled out by the experiment.

The latest of this series of experiments (Bateson, Horn & Rose 1975) used only trained birds. There is great variability between birds from the same hatch in their behaviour towards the imprinting stimulus; some respond very early in the exposure period and others much later; some develop a weak and others a strong preference for the familiar stimulus. A large number of measures of the birds' behaviour, during both exposure and testing, were recorded and we examined their degree of correlation with the extent of incorporation in four brain regions. There were precursor incorporation effects (depressions) in the whole brain which correlated with the latency of response to the stimulus, and a just-significant correlation between specific radioactivity in the anterior forebrain roof and preference for the familiar, the measure of learning used in these experiments. When the depressions were accounted for by relating the incorporation in the two portions of the forebrain roof to that in the forebrain base and midbrain there remained only one significant correlation, namely between the incorporation in the anterior forebrain roof and the bird's preference for the familiar as opposed to a novel stimulus. To put it simply, the more the birds learned, the more uracil they incorporated into RNA in the anterior forebrain roof. No other biochemical measure among those we made could be related to an elevation in incorporation.

These behavioural controls are not, of course exhaustive. For example, it could be argued that both the birds propensity to learn and to incorporate uracil into RNA are consequences

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of a quite separate prior effect – for example ontogenetic or other differences in 'attention' or 'motivation'. And of course the fact that *one* of our biochemical measures shows no correlation except with learning does not logically allow the inference that the same is true for all the others, whether the protein incorporation or the enzyme changes. Indeed, the balance of probability suggests that some of the biochemical changes may indeed be correlates of more general phenomena.

We do however claim that, taken together, our imprinting experiments have got us to the point where we can embark on the third phase of our strategic approach – the identification of the precise sequence of biochemical changes, and their anatomical localization, which represent the necessary, sufficient and exclusive correlates of imprinting.

Conclusions

It is not appropriate here to present a general theoretical framework for interpreting phenomena of plasticity and learning; this has been discussed elsewhere (Horn *et al.* 1973*b*; Rose 1973; Rose *et al.* 1975), and must take into account the many other studies in this area which the present paper, by concentrating almost exclusively on our own work, has omitted.

We believe we have shown that one consequence of visual deprivation in the rat is the suppression of a portion of neuronal protein synthesis in the visual cortex, that on light exposure the rate of production of a number of protein fractions is enhanced, and that some at least of these proteins are likely to be particulate glycoproteins which form part of the cellulofugal export for which the neuronal cellular machinery is partially specialized. An obvious inference is that the proteins concerned may have some role in the modulation of connectivity at either the pre- or post-synaptic sites, and we are at present testing just this possibility.

What type of cellular events precede the elevated synthesis? If the chick data may be combined with that of the rat experiments, we may suggest an elevated RNA polymerase and an enhanced production of m-RNA. What signals, presumably at the cell surface, and what types of 'second messenger' may be involved in instructing the molecular mechanisms concerned, can at present only be a matter for informed molecular biological speculation.

As to the cellular role of the new proteins, we assume, at least as a hypothesis to be tested, that they would have a role in modulating connectivity as one aspect of the nervous system's long-term plastic modification in response to experience. We may also refer to the apparently transient changes in enzyme activity that we have observed in both rat and chick systems, and suggest that these are part of the short term response of the system to experience which may be the correlate of any one or more of the several behavioural processes which are attendant upon learning.

Finally, we may ask whether those changes we have observed have anything to do with learning itself. There remains doubt on this of course, but we feel that, in the chick imprinting situation, we have progressively eliminated most, but not all of the other possibilities. When our data is taken in conjunction with the other biochemical approaches to the cellular changes involved in learning, such as the inhibitor studies (reviewed in Rosenzweig & Bennett 1975), we may feel reasonably confident that there is a close relationship between an enhanced rate of synthesis of certain specific proteins in particular areas of the brain, and learning. Such biochemical changes may depend upon the same types of cellular and molecular responses as in more general forms of long term nervous system plasticity, but involving a relatively small

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number of cells and their connections. We believe that the way is now open to a precise identification of just which cells, which types of protein and which cellular functions are involved.

Addendum

Since this review was written, we have been able to identify one of the protein fractions whose synthesis is suppressed in the visual cortex of dark-reared rats and stimulated by light exposure, as tubulin, the major component of microtubules. Tubulin synthesis in the dark-reared animals is only 50 % of normal, but is doubled within an hour of exposure to the light (Rose, Sinha & Jones-Lecointe 1976).

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