SYNTHESIS OF TUBULIN-ENRICHED FRACTION IN RAT VISUAL CORTEX IS MODULATED BY DARK-REARING AND LIGHT-EXPOSURE

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Received 13 April 1976

1. Introduction

Microtubules are known to have an important role in the brain, especially in relation to the transport of materials from the nerve cell body to synaptic sites [1]. The principal component of microtubules is the polymerisable protein, tubulin. In this paper, we report evidence that the rate of synthesis of a polymerisable tubulin-enriched protein fraction in the visual cortex of the rat brain is reduced during dark-rearing and is enhanced by exposing the dark-rearing animals to light.

In previous experiments [2] we have shown that the rate of protein synthesis in the neurons of the visual cortex of dark-reared rats is lowered compared to that in their normally-reared littermates [3]. When rats reared for 50 days in the dark are exposed to light, there is an increased protein synthetic rate in the visual cortex, lateral geniculate and retina [4]. An hour after onset of illumination and injection of radioactivity, most of the increased protein radioactivity is bound to the ribosomes or in a small number of soluble fractions [5]. Because of the known importance of tubulin in the neuronal economy [6,7] it seemed sensible to see if any of the soluble protein fractions whose synthetic rate was modulated by dark-rearing and light-exposure could be related to tubulin.

2. Experimental

The experimental procedure was similar to that of our earlier experiments [3–5]. Male Wistar rats were born and maintained in the dark until weaning (21 days). One-third were then placed in a normal 12 h light/12 h dark animal house cycle (normals: N), and the remaining two-thirds returned to the dark until 45–50 days of age. Half the dark-reared animals were then brought out into the light (light-exposed: L) and the remainder stayed in the dark (dark: D). Ns, Ls and Ds were injected intraperitoneally with 100 μCi [^{3}H]lysine (18 Ci/mmol.; Radiochemical Centre, Amersham) in 0.5 ml 0.9% saline, and killed after 1 h of exposure to their respective conditions. Visual and motor cortices were dissected out and processed individually.

For purification of polymerised tubulin, the first stage of the microtubule assembly method of Shelanski et al. [8] was used to prepare a tissue homogenate (H), and two primary fractions, a 100 000 *g* pellet (P_1) and supernatant (S_1). Tubulin was polymerised from S_1 in the 8 M glycerol-reassembly buffer and separated by centrifugation for 1 h at 140 000 *g* yielding a pellet P_2 and supernatant S_2. According to Shelanski et al., the P_2 pellet is 75–85% purified tubulin. Protein and radioactivity were measured in H, P_1, S_1, P_2, and S_2 and specific radioactivity (dpm/mg protein) was calculated exactly as described previously [3–5]. A portion of the P_2 fraction was routinely taken for electron microscopy.

3. Results and discussion

Shelanski et al. used a tissue/reassembly buffer volume ratio of about 1/1 in their initial homogenisation, and their P_2 pellet contained 11–15% of the S_1 protein. Using a similar tissue/buffer ratio in our preliminary experiments, protein recovery in P_2 was

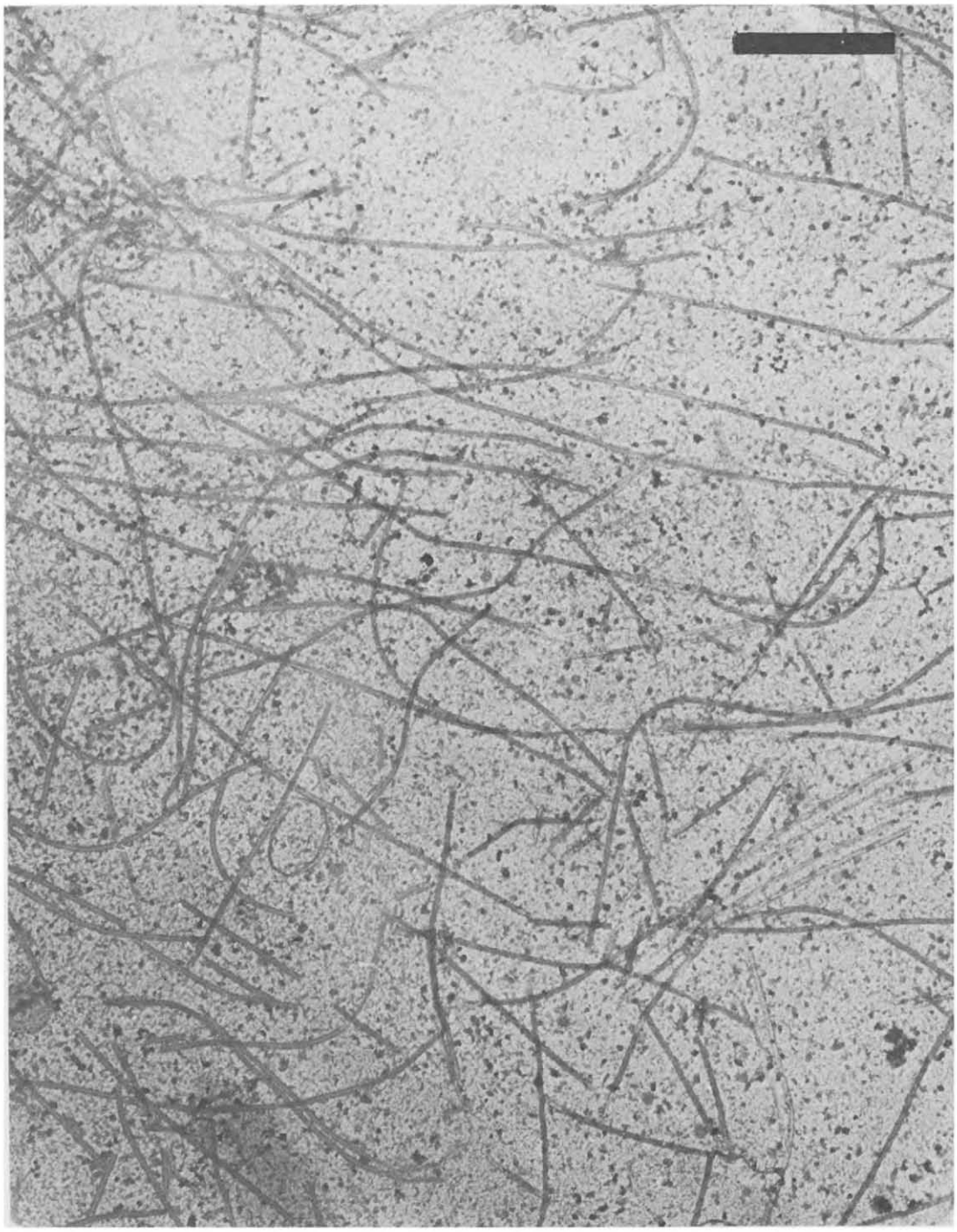

Fig.1. Electron micrograph of P_2 fraction. An aliquot of the fraction was dried on a carbon-coated grid, negatively stained with 1% uranyl acetate and observed using a Corinth 275 electron microscope. Bar = 1 μm.

12.5 ± 0.7% of S_1, whilst ^3H recovery was 11.4 ± 0.3%. This represented approx. 4% of the total protein and radioactivity in the initial homogenate, and the specific radioactivity (dpm/mg prot.) of the P_2 pellet was 90% of that of H. Thus [^{3}H] lysine as a precursor was incorporated into the tubulin-enriched fraction at a rate not very different from the average for all proteins under these conditions. However, both the protein distribution between P_1 and S_1 and between P_2 and S_2 are rather critically dependent on the tissue/buffer volume ratio [9] and in our experiments we wished to treat each visual and motor cortex sample, consisting of 5–10 mg protein, separately. This meant homogenisation using a tissue/buffer ratio as much as 1/30 and preliminary experiments showed that this reduced the proportion of protein both in S_1 and in P_2. P_2 was now 7.5 ± 0.4% of S_1 and only 1.5 ± 0.3% of H. (There were no significant differences in P_2 protein as a proportion of H in either brain region in any of the experimental conditions.) The amount of recovered radioactivity in P_2 was lowered in parallel to the protein. Electron micrographs of the P_2 fraction showed a characteristic microtubular appearance (fig.1) and thus although the yield of P_2 from the visual and motor cortex samples was reduced, its identification as largely consisting of polymerised tubulin seems secure.

In the behavioural experiments, seven experimental groups each composed of 1N, 1L and 1D animal were used, together with a further group containing 2N and 1D. To account for batch variation between experimental groups in body weight, injection procedure, etc, two methods of calculation were used. In the first, mean specific radioactivities for each fraction were calculated for each experimental group and normalized around a mean specific radioactivity in the homogenate of 1000 dpm/mg protein. In the second, the specific radioactivity ratios in L/N, D/N and L/D were calculated for each fraction in each experimental group. Tests of significance were made using Student's t. The normalized specific radioactivities for the visual cortex are given in table 1 and significant differences for the comparisons identified in the caption. There were no significant differences, or trends approaching significance, for any fraction or condition in the motor cortex. All observed effects thus showed a regional specificity.

Table 1

Normalised specific radioactivities of protein fractions in visual cortex, of normal, dark-reared and light-exposed animals

Fraction	dpm/mg prot.		
	N	L	D
H	1009 ± 68	1211 ± 130	846 ± 45
P_1	791 ± 58	1069 ± 137	714 ± 55
S_2	1041 ± 54	1021 ± 83	954 ± 109
P_2	1073 ± 103	1158 ± 72	580 ± 33

Normalised means ± s.e.m. from 8 separate triplet-group experiments involving 7L, 8D and 9N animals. Nomenclature of fractions and experimental details are as in the text. Comparing L versus D, D versus N and D versus L for each fraction and each brain region, the following differences are significant:

H; D versus L $t = 2.80$, $p < 0.02$
(D versus N $t = 1.95$, n.s.)

P_1; D versus L $t = 2.53$, $p < 0.02$

S_2; nil

P_2; D versus N $t = 4.34$, $p < 0.001$
D versus L $t = 4.31$, $p < 0.001$

The elevation of 43 ± 17% of L over D in the homogenate specific radioactivity essentially replicates our earlier observations [4]. In the P_1 fraction, L showed a tendency to be higher and D to be lower than N, and the significant difference was a 50 ± 22% elevation in incorporation in L compared with D. This too is compatible with our earlier observation that there is very much enhanced incorporation into a ribosomally bound fraction in the visual cortex of L animals [5], as under these conditions of homogenisation and centrifugation, ribosomes will appear in the P_1 fraction. There were no significant differences in specific radioactivity between conditions in the S_2 fraction (nor in the S_1 fraction from which S_2 and P_2 are derived). However, there was a large and highly significant reduction in specific radioactivity in the P_2 polymerised tubulin fraction in the dark visual cortex compared with Normals or Lights. The ratios D/N and L/D are shown for P_2 for visual and motor cortex in each of the seven individual experiments in table 2, indicating both the consistency of trend and range of variability of the effect. Compared to the Darks, there was an average doubling of P_2

Table 2
Comparison of specific radioactivities of tubulin-enriched fractions from visual and motor cortex of normal, dark- and light-exposed animals

Ratio in P_2		Experiment No. 1	2	3	4	5	6	7	Mean ± s.e.m.	p
Visual cortex	N/D	2.44	1.59	0.99	1.75	1.96	1.64	2.00	1.64 ± 0.19	< 0.01
	L/D	1.23	1.35	1.60	2.41	2.29	3.19	2.64	2.10 ± 0.28	< 0.01
Motor cortex	N/D	0.92	1.92	–	1.64	0.89	0.55	1.79	1.29 ± 0.23	ns
	L/D	0.99	1.64	–	1.28	0.56	0.40	2.17	1.17 ± 0.27	ns

Ratios of specific radioactivities in P_2 fraction from the visual and motor cortex in normal and light-exposed animals compared with dark, for 7 experiments in each of which a triplet of 1N, 1L and 1D animal were used. Motor cortex sample for D in experiment 3 is missing. These experiments are included in the specific radioactivity data given in table 1.

specific radioactivity in the Light visual cortex. Again, despite considerable variability there were no significant differences in ratios between Lights, Darks or Normals in the motor cortex.

We conclude that incorporation of precursor into, and hence probably the synthesis of, a tubulin-enriched fraction is lowered in the visual cortex of dark-reared rats. On exposure to the light, there is a substantial increase in the synthesis of P_2 protein resulting in a doubling of the incorporation rate in the first hour of light exposure. Whilst the P_2 fraction contains minor components other than tubulin, it would seem unlikely that a change of this magnitude in the specific radioactivity of the whole fraction could occur as a result simply of a change in such minor components, as this would require a many-fold increase in their specific radioactivity. The rapidity with which increased incorporation into the tubulin-enriched fraction occurs following onset of light exposure is striking.

In earlier experiments we have shown that one of the proteins whose synthesis is lowered in the visual cortex of dark maintained animals, and enhanced on light exposure, is a rapidly labelling and exported neuronal protein, whose export is blocked by colchicine [10] and we have argued that this made it likely that some component of the flow of protein from neuronal cell body to axon and dendrites was subject to environmental modification. The present result strengthens this conclusion by showing the sensitivity of a key protein involved in the structure and metabolic regulation of dendrites, axons and synapses, to environmental change. Fellous et al. [11] have claimed that brain tubulin levels vary markedly during early development in the rat, while Cronley-Dillon and Perry, in a brief report, have stated that both the amount and rate of synthesis of tubulin increase substantially in the visual cortex in the period around eye opening in the rat [12], but it is not clear from their observation whether this increase is dependent upon functional stimulation. The experiments reported here suggest that the rate of tubulin production in the visual cortex is indeed related to functional stimulation.

Acknowledgements

We thank Dr J. R. Lagnado of Bedford College for advice concerning the purification of tubulin, L. Sinha for technical assistance, D. Spears for electron micrography, T. Hedges and S. Walters for animal maintenance and Dr J. Schwartz and other members of the Brain Research Group for critical discussions.

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