Development of Isolated Neocortex

It has been suggested that the thalamocortical fibres present in developing neocortex may form the substrate along which neuroblasts migrate from the ventricular zone to the subpial cortical plate[1]. Corticopetal fibres may also be implicated in the organisation of cortical laminae[2,3] and in the growth of neocortical dendritic fields[4–8]. These contingencies have been investigated by sectioning the corona radiata in the brains of foetal rats and studying the subsequent development of the isolated cortex.

Materials and methods. Thalamocortical input to the neocortex of Wistar rats was surgically interrupted on the 16th, 17th, 18th, 19th, 20th, 21st, 23rd and 25th days post conception (p.c.). Rats were usually born on the 23rd day p.c. Operations in utero were performed by inserting a fine stainless steel wire (diam. 125 µm), supported in the barrel of a hypodermic needle, into the frontal pole of the lateral ventricle of the foetal brain while transilluminating the uterus. The tip of the wire was pushed into the occipital pole of the foetal cortex and withdrawn with the tip held gently against the uterine wall. This procedure severed the cortex along a line from the occipital to the frontal poles parallel and lateral to the sagittal fissure. By similar means an identical incision was placed in the cortex of neonatal animals. The migration of neuroblasts was studied in the isolated cortex by labelling with tritiated thymidine[9] (H^3T) at the time of section (Group A – 40 animals) or at of 2 days (Group B – 40 animals) or 4 days (Group C – 40 animals) after section. In each group 1 animal was sacrificed on the 2nd, 4th, 6th, 8th and 10th days after injection of H^3T. The development of the dendritic tree of neocortical neurons was studied at 30 days post partum in normal rats (16 animals) and after thalamocortical section on the 19th (12 animals), 21st (11 animals), 23rd (8 animals) and 25th (8 animals) days p.c. Using the target method of EAYRS[10], the density of the basal dendritic networks of Golgi-Cox impregnated pyramidal neurons in layers IV and Vb of the sensorimotor cortex were measured using celloidin sections (150 µm thick) cut in a standard coronal plane[11]. The size of dendritic fields was estimated from the total

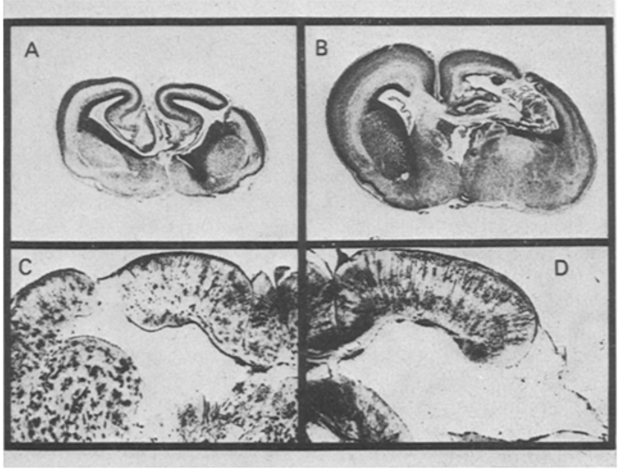

Fig. 1. Coronal sections of the brains of rats; A, aged 20 days p.c., isolated on the 16th day p.c.; B, aged 22 days p.c. isolated on the 17th day p.c. (Nissl, ×16). In both cases note the reduction in thickness of the cortex on the operated side. C and D coronal sections of the brains of rats aged 30 days post partum after isolation on the 19th and 21st days p.c., respectively. Note the completeness of isolation of the cortex from the diencephalon and the presence of the corpus callosum (Golgi-Cox impregnations, ×16).

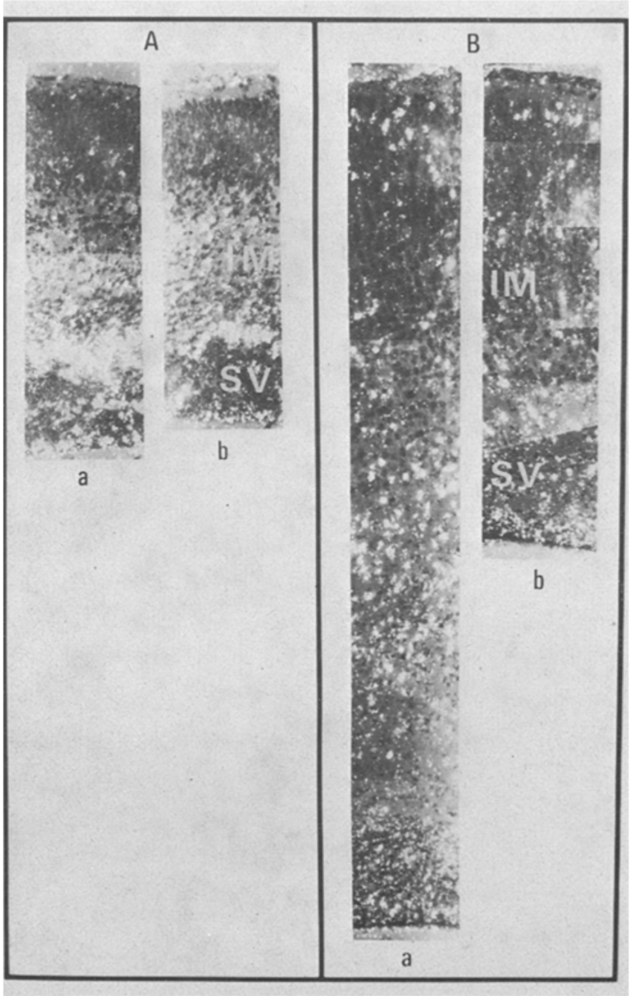

Fig. 2. A, detail of the neocortex of the specimen shown in Figure 1a, aged 20 days p.c., a, normal cortex and b, contralateral cortex isolated on the 16th day p.c., following the administration of H_3T on the 18th day p.c. B, detail of the neocortex of the specimen shown in Figure 1b aged 22 days p.c., a, normal cortex and b, contralateral cortex isolated on the 17th day p.c., following the administration of H_3T on the 19th day p.c. In both cases note the reduction in thickness on the operated side mainly affecting the subventricular (SV) and intermediate zone (IM) and the normal migration of labelled neuroblasts (Autoradiograph-photographed in both transmitted and incident lighting — Nissl × 352).

[1] S. P. HICKS and C. J. D'AMATO, Anat. Rec. *160*, 619 (1968).
[2] M. MARIN-PADILLA, Brain Res. *23*, 167 (1970).
[3] M. MARIN-PADILLA, Z. Anat. EntwGesch. *134*, 117 (1971).
[4] P. D. COLEMAN and A. H. RIESEN, J. Anat. *102*, 363 (1968).
[5] R. L. HOLLOWAY, Brain Res. *2*, 393 (1966).
[6] M. C. DIAMOND, F. LAW, H. RHODES, B. LINDNER, M. R. ROSENZWEIG, D. KRECH and E. L. BENNETT, J. comp. Neurol. *128*, 117 (1966).
[7] A. GLOBUS and A. B. SCHEIBEL, Exptl Neurol. *19*, 331 (1967).
[8] W. H. JONES and D. B. THOMAS, J. Anat. *96*, 375 (1962).
[9] M. BERRY and A. W. ROGERS, J. Anat. *99*, 691 (1965).
[10] J. T. EAYRS, Acta anat. *25*, 160 (1955).
[11] J. T. EAYRS and B. GOODHEAD, J. Anat. *93*, 385 (1959).

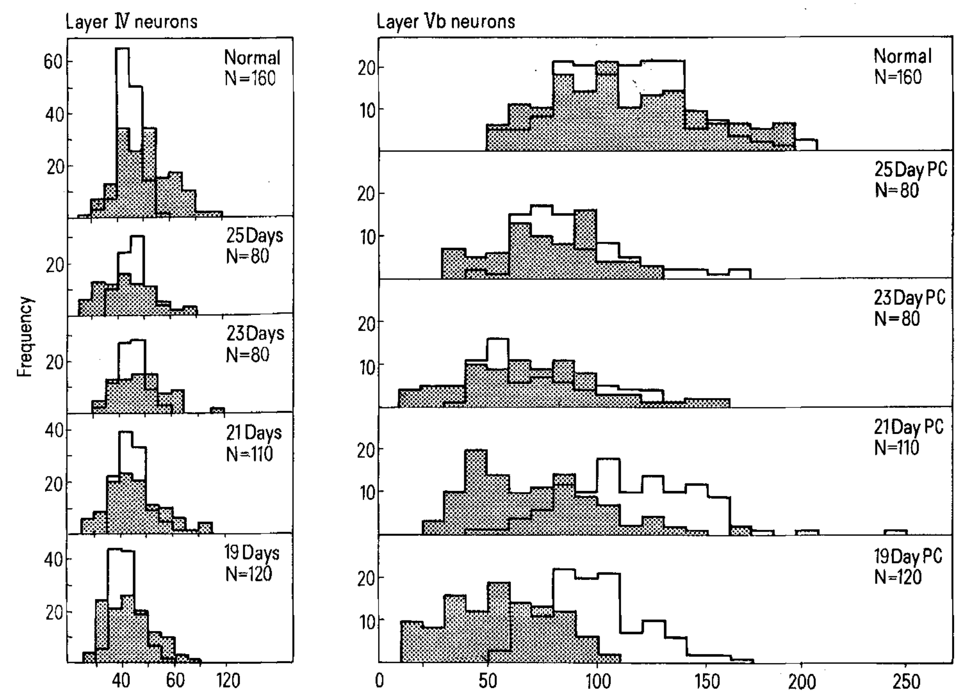

Fig. 3. Frequency histogram of the number of intersections/neuron and the size of the soma of neurons in layer IV and Vb in normal cortex and cortex isolated on the 19th, 21st, 23rd and 25th days p.c. Note the increasing dissociation of dendritic density and the size of the soma in layer Vb the earlier the operation is performed and the normal association of dendritic density and the size of the soma in the neurons of layer IV. □ Area of soma arbitrary units. ▦ Number of intersections.

Values for the sizes of the soma and densities of dendritic fields of neurons in layer IV and Vb of normal cortex and cortex isolated on the 19th, 21st, 23rd and 25th days p.c. – see text.

Property	Layer	A Normal animals ($N = 16$)	B 19th day p.c. animals ($N = 12$)	C 21th day p.c. animals ($N = 11$)	D 23th day p.c. animals ($N = 8$)	E 25th day p.c. animals ($N = 8$)	Value of P A and B	unpaired A and C	t comparisons between A and D	 A and E
1. Area of soma (A)	IV	46.7 ± 4.6	43.2 ± 5.8	48.7 ± 6.8	49.1 ± 4.5	52.3 ± 7.1	0.3 – 0.2	0.3 – 0.2	0.3 ± 0.2	0.05 – 0.02
2. Total No. of intersections (I)	IV	60.3 ± 11.4	45.3 ± 12.4	51.3 ± 12.4	56.2 ± 9.3	46.8 ± 12.2	0.01 – 0.001	0.1 – 0.05	0.4 ± 0.3	0.02 – 0.01
3. I/A %	IV	129.6 ± 27.0	107.4 ± 31.5	108.0 ± 32.2	115.1 ± 22.7	89.5 ± 23.8	0.05 – 0.02	0.05 – 0.02	0.3 – 0.2	0.01 – 0.001
4. Area of soma (A)	Vb	110.8 ± 14.4	94.6 ± 13.5	115.9 ± 12.0	105.2 ± 14.0	87.5 ± 9.9	0.01 – 0.001	0.4 – 0.3	0.4 – 0.3	< 0.001
5. Total No. of intersections (I)	Vb	114.4 ± 21.8	69.0 ± 18.8	71.2 ± 18.9	89.8 ± 17.0	76.9 ± 17.3	< 0.001	< 0.001	0.02 – 0.01	< 0.001
6. I/A %	Vb	102.9 ± 13.0	72.6 ± 14.2	61.4 ± 14.6	85.7 ± 11.8	88.0 ± 18.8	< 0.001	< 0.001	0.01 – 0.001	0.05 – 0.02

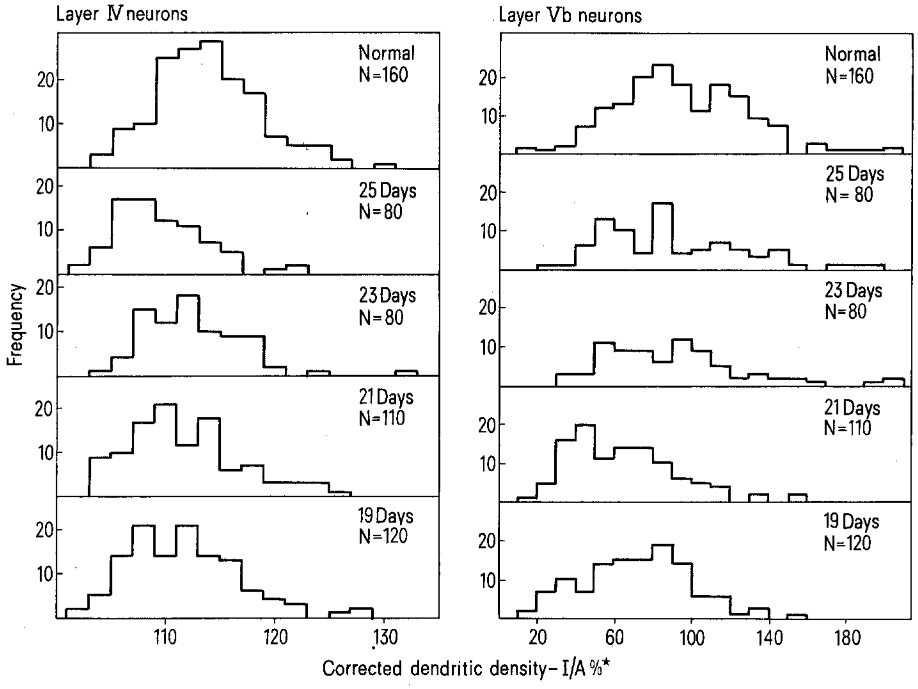

Fig. 4. Frequency histograms of the corrected dendritic density (*number of intersections/neuron/area of soma %) of neurons in layer IV and Vb of normal cortex and cortex isolated on the 19th, 21st, 23rd and 25th days p.c. See text for full explanation.

number of intersections/target by summing the numbers of dendrites intersecting each of the 10 concentric circles of the target. The size of the soma was measured by weighing the paper cut-outs of the silhouettes of Golgi-Cox impregnated perikarya after × 3 photographic enlargement. Dendritic fields were corrected for the size of the soma and compared by Student's t-testing. This latter correction is necessary since the size of the soma and dendritic density are directly and positively related[12].

Results. Although the operation induced some degree of ipsilateral hydrocephalus the isolated piece of cortex showed no necrosis even at the margins of the incision (Figure 1). A reduction in the thickness of isolated cortex occurred which appeared to be attributable to a local reduction in the thickness of the subventricular and intermediate zones and to hypoplasia of the cortical plate (Figures 1 and 2). The migration of neuroblasts proceeded normally at all the time intervals studied (Figure 2) and established the normal sequence of lamination without delay. The size of both the dendritic fields and the soma of the neurons of layer Vb in isolated cortex was, in most cases, reduced (Table, Figure 3) and this reduction was maintained after correcting for the size of the soma (Table, Figure 4). The effects of deafferentation on the neurons of layer IV were different. Cell body size was not reduced (Table, Figure 3) although dendritic density showed a decrement in all experimental groups (Table, Figure 4). There was no qualitative difference between the geometries of dendritic fields of neurons in normal and isolated cortex. The corpus callosum had formed in all animals.

Discussion. The absence of corticopetal fibres and the death and possible shrinkage of neocortical efferent cells following section of corticofugal fibres (Gudden effect[13]) may, in the main, account for the reduced thickness of isolated cortex when compared with the normal. It is unlikely that the blood supply to the isolated piece of cortex was in jeopardy at any time after section[14]. The normal migration and subsequent lamination of neuroblasts into the 6 layers of the neocortex, in the absence of thalamocortical fibres, favours the thesis that cortical histogenesis and the movements of neuroblasts are organised by intrinsic[15] rather than extrinsic[1] factors. Although the effects of section of the corona radiata on the neurons of layer Vb may be complicated by the Gudden effect the analysis of the growth of dendritic fields after deafferentation suggests that extrinsic factors affect dendritic networks quantitatively rather than qualitatively despite the presence of callosal fibres and their possible influence on developmental processes[16]. This result is in agreement both with the observations of Van der Loos[17], that the geometry of improperly orientated dendritic fields develops normally notwithstanding their altered environment, and with the finding of Coleman and Riesen,[4] that deafferentation in the adult may cause a reduction in the size of dendritic fields. The method of analysis used in this study was not sufficiently sensitive[18] to detect the changes in orientation of dendrites which have been shown to occur in the neurons of layer IV following the deprivation of specific sensory input[19].

[12] M. Berry, T. Hollingworth, R. Flinn and E. M. Anderson, *Macromolecules and Behaviour* (Macmillan, London, in press).

[13] A. La Velle and F. W. La Velle, Exptl Neurol. *1*, 82 (1959).

[14] L. H. Strong, J. comp. Neurol. *123*, 121 (1964).

[15] P. Rakic J. comp. Neurol. *145*, 61 (1972).

[16] M. Marin-Padilla, Z. Anat. EntwGesch. *136*, 125 (1972).

[17] H. Van der Loos, Bull. Johns Hopkins Hosp. *117*, 228 (1965).

[18] M. Berry, T. Hollingworth, R. Flinn and E. M. Anderson, T. I. T. J. Life Sci., in press.

[19] F. Valverde, Exptl Brain Res. *5*, 274 (1968).

Résumé. La migration des neuroblastes et la croissance des aires dendritiques de base des neurones pyramidaux font ici l'objet d'une étude portant sur les régions IV et Vb du néocortex cérébral isolé de rats de souche Wistar. La migration des neuroblastes s'éffectuait normalement et aboutissait au dispositif habituel ordonné en 6 régions. Aucun changement qualitatif ne fut observé dans la méthode de croissance dendritique des cortex cérébraux isolés, mais la densité des aires dendritiques était diminuée dans chaque cas, bien que les effets sur les neurones de la région Vb fussent rendus complexes par l'effet de Gudden.

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Neurosecretory Flow in the Perioesophageal Tract of *Jonespeltis splendidus* (Diplopoda, Myriapoda)

In the millipede, *Jonespeltis splendidus*, dense neurosecretory material is present in the brain, arranged in the form of a perioesophageal arc. The C_1 cells distributed along the ventral and mesial side of the brain elaborate this material, the connective bodies being the neurohaemal organs of this tract[1,2]. A similar neurosecretory tract extending into the suboesophageal ganglion has been described in some other diplopods[3,4], in which migration of the neurosecretory material from the suboesophageal ganglion to the brain via the lateral oesophageal connectives is thought possible[4]. The present investigation experimentally verifies the course of the neurosecretory material in this tract.

Materials and methods. Adult males and females of the millipede *Jonespeltis splendidus*, of body length 25–30 mm, collected from field were kept in the laboratory on decaying leaves and vegetables with plenty of humus on which they fed. Severance of the connective of one side was effected either above or below the level of the connective body by dorsal approach[5], that of the other side being kept as control. Of the 100 animals operated upon, about 50% survived. Animals were sacrificed at 5 day intervals up to 1 month. The circumoesophageal ring was dissected out and processed as a whole[6]. Some 5 µm sections of the nerve ring fixed in Bouin's fluid were also stained for neurosecretion[7,8].

Results and discussion. These studies revealed the perioesophageal tract of neurosecretory material ending mostly in the connective bodies and partly extending into the suboesophageal ganglion (Figures 1 and 2) as observed in other diplopods[3,4]. Unoxidized preparations which were processed as controls did not reveal the stainable material. In addition, C_1 and C_2 neurosecretory cells[1] revealed cystine/cysteine-rich material. None of the other neurosecretory cells or tracts of the cerebral glands[2] were stainable with these techniques, obviously because they contained no cystine/cysteine-rich material. In those animals where the lateral oesophageal connective was severed above the level of the connective body, the stump of the connective closer to the brain showed gradual accummulation of stainable material in the neurosecretory axons, but not in other fibres. Since in this case the connective body of the severed side was depleted of almost all the stainable material whereas that

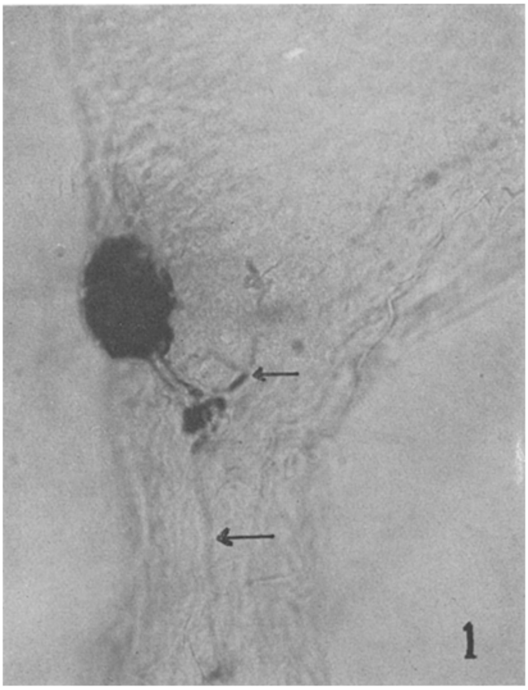

Fig. 1. Whole mount of the brain of *Jonespeltis* showing the neurosecretory tracts from the brain (upper arrow) and from the suboesophageal ganglion (lower arrow) entering the connective body (CB). Performic acid/Victoria blue staining. ×250.

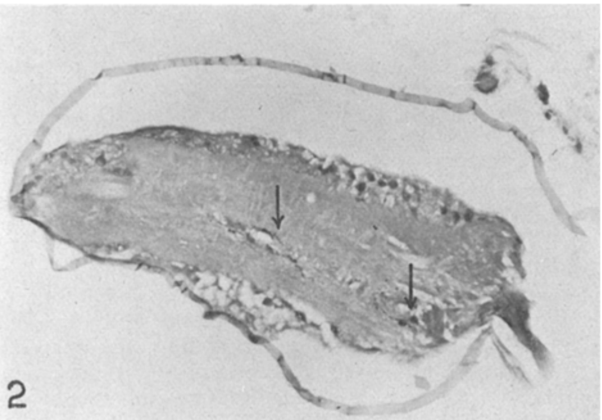

Fig. 2. Neurosecretory tract (arrows) of the suboesophageal ganglion in parasagittal section, fixed in Bouin and stained in chromealum haematoxylin phloxin.

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[1] V. K. K. Prabhu, Z. Zellforsch. *54*, 717 (1961).

[2] V. K. K. Prabhu, in *Neurosecretion* (Mem. Soc. Endocrin. 1962). vol. 12, p. 417.

[3] F. Sahli, C.r. Acad. Sci., Paris *254*, 1498 (1962).

[4] F. Sahli, Thèse (Paris 1966), p. 228.

[5] V. K. K. Prabhu, Ind. J. exp. Biol. *2*, 5 (1964).

[6] G. S. Dogra and B. K. Tandon, Q. Jl microsc. Sci. *105*, 455 (1964).

[7] M. L. Cameron and J. E. Steele, Stain Tech. *34*, 265 (1959).

[8] G. Gomori, Am. J. Path. *17*, 395 (1941).