Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Prenatal Development of the Visual System in Rhesus Monkey

P. Rakig

Phil. Trans. R. Soc. Lond. B 1977 **278**, 245-260
doi: 10.1098/rstb.1977.0040

References Article cited in:
http://rstb.royalsocietypublishing.org/content/278/961/245#related-urls

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1977 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

Phil. Trans. R. Soc. Lond. B. **278**, 245–260 (1977) [245]
Printed in Great Britain

Prenatal development of the visual system in rhesus monkey

By P. Rakic
Department of Neuropathology, Harvard Medical School and Department of Neuroscience, Children's Hospital Medical Center, Boston, Massachusetts 02115, *U.S.A.*

[Plates 1–4]

Autoradiographic evidence from juvenile rhesus monkeys that had been exposed to a pulse of [^{3}H]thymidine at different embryonic (E) and early postnatal (P) days indicates that all neurons which compose the visual system of this primate have been generated two months before birth. The first retinal ganglion cells (RGC) are generated around E30 preceding by a few days the onset of genesis of neurones destined for the dorsal lateral geniculate body (LGd) and superior colliculus (SC) both of which begin at E36. Production of neurons destined for the primary visual cortex (area 17) begins at approximately E43 and ends by E102. Neurons destined for layer IV, the major target of axons from the LGd, are generated between E70 and E85.

The prenatal development of visual connections was studied by the autoradiographic method of anterograde axoplasmic transport in foetuses killed 14 days after unilateral eye injection of a mixture of [^{3}H]proline and [^{3}H]fucose. Initially, in the LGd and in the SC projections from both eyes overlap. Segregation of the axons and/or terminals from the two eyes occurs in the LGd and SC during the middle period of gestation. Transneuronal transport of tritium shows that although LGd axons form the optic radiation before E78, these fibres do not yet enter the developing cortical plate at this foetal age. During the second half of gestation, geniculocortical axons carrying input from each eye invade the cortex but are not yet segregated into ocular dominance columns. Rather, grains are distributed uniformly over the entire layer IV at E124. Three weeks before birth, at E144, segregation of afferents into sub-layers IVA and IVC is apparent, and the first hint of ocular dominance columns is displayed by slight differences in grain counts in alternating areas of layer IV.

These results show that all neurons in the primate visual system have been generated, reached their final positions and formed their basic connections subserving ocular dominance before birth, i.e. before visual experience. In the SC and LGD, monocular segregation is well established during the middle period of gestation, whereas in the cortex it has begun, but is not fully developed at birth.

1. Introduction

Perhaps there is no more appropriate topic to begin a meeting on structural and functional aspects of plasticity than one which deals with the analysis of neuronal events that occur during development. The orderliness and regularity of the cellular organization and pattern of synaptic circuitry in the adult central nervous system is the end result of an enormous number of changes that occur during ontogeny. Individual neurones change their position relative to one another; many of them migrate long distances to attain their final locations; their processes become redistributed while entire brain structures are shifted, and synaptic junctions change their size and location on target cells. In this report, I will present some new evidence that specific patterns of neuronal connectivity in the adult visual system are achieved through several transient stages that become modified by subsequent developmental events.

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

The rhesus monkey is an excellent species for the study of the development of neuronal connections in the visual system. The anatomy and physiology of the visual system in this animal is relatively well-established (e.g. see Polyak 1957 for old and Jung 1973 for recent reviews). As schematically illustrated in figure 1, axons of the retinal ganglion cells from each eye partially cross at the optic chiasma (CH) and project to the 6 laminae of the dorsal lateral

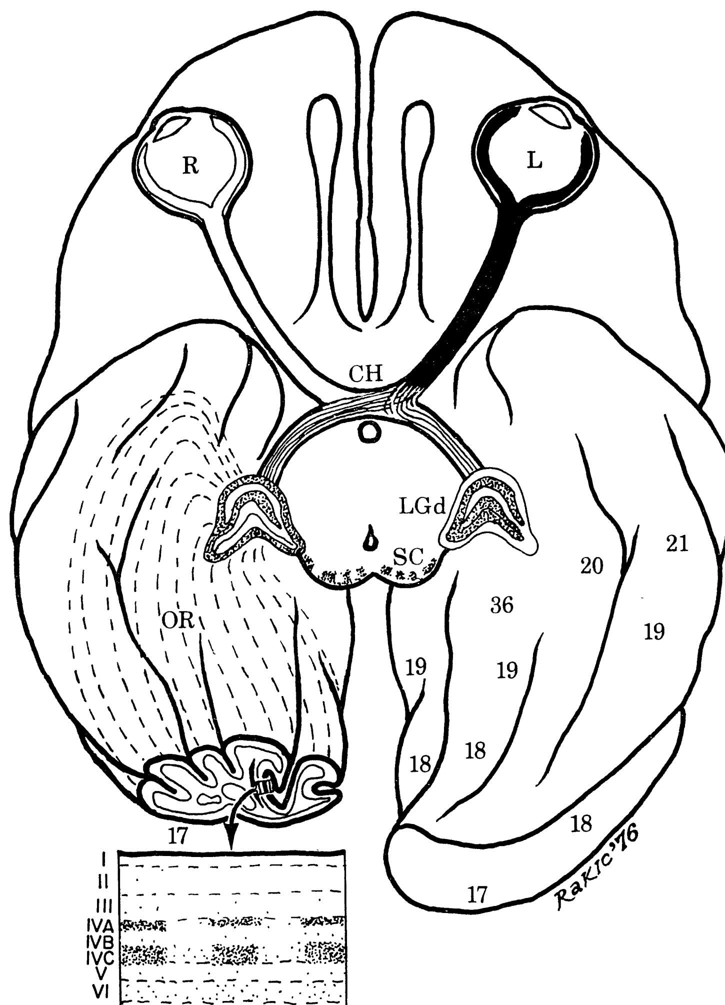


FIGURE 1. Semi-schematic illustration of the connections underlying binocular vision in the rhesus monkey. On the brain viewed from below, the dorsal lateral geniculate body (LGd) and superior colliculus (SC) are slightly enlarged to render the details of binocular representation legible. For the same reason, a small region of area 17 in the depth of calcarine fissure is enlarged in the left lower corner of the diagram (curved arrow). The axons originating from retinal ganglion cells of each eye partially cross at the optic chiasma (CH) and are distributed in the 3 appropriate laminae of the LGd and to the appropriate territories representing each eye in the SC. Principal neurons of the LGd project to the primary visual cortex (area 17) via the optic radiation (OR) and terminate mostly in sublayers 4A and 4C in the form of alternating columns that receive input from one or the other eye.

geniculate body (LGd). More than 55 years ago Minkowski's (1920) studies of human material demonstrated that three laminae (1, 4 and 6) of the LGd receive direct inputs from the contralateral eye and the remaining three (2, 3 and 5) from the ipsilateral eye. Although it has been known for some time that the laminar organization of the LGd in monkey is similar to that of man, a particularly elegant demonstration of the retinogeniculate projections has

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

been recently provided by the method of autoradiography following injections of radioactive proline and fucose into one eye (Wiesel, Hubel & Lam 1974). The autoradiographic method also provided the first anatomical evidence for segregated representation of each ocular field in the superior colliculus (SC), where the fibres from the two eyes terminate in complementary, alternating territories 0.1–0.5 mm wide (Hubel, LeVay & Wiesel 1975). Furthermore, since a small but demonstrable amount of radioactivity is transported transneuronally beyond the LGd (Grafstein 1971), label can also be found in the primary visual cortex (area 17) after ocular injection of tritiated amino acids and sugars. Following injections into one eye, the label in the monkey primary visual cortex is concentrated in two strata corresponding to sublayers IVA and IVC (Wiesel *et al.* 1974), as 350 μm wide bands that alternate with bands of the same width containing low grain counts (figure 1, enlarged square of the area 17). These results basically confirm previous anatomical (Matthews, Cowan & Powell 1960; Hubel & Wiesel 1972) and physiological studies (Hubel & Wiesel 1968) on the organization of the binocular system in the monkey visual system, and provide a simple and reliable method for exploring when the separation of inputs from each eye occurs in the SC, LGd and primary visual cortex.

The present study deals with several related questions: Are the structural organization and connections subserving the binocular system established before birth? If so, are the inputs from the left and right eye already separated at the time when retinal fibres begin to invade the analage of the LGd, SC and cortical plate or, alternatively, do fibres become segregated secondarily? Is the laminar organization of visual centres governed by intrinsic factors or is it dependent on the pattern of its afferent input? Is the mechanism of formation of visual connections the same for subcortical and cortical visual centres? The large size of primate brain and protracted span of macaque development (gestation is 165 days long) increases the resolution of spatio-temporal sequences during neurogenesis and thus offers advantages over more readily available species for the study of some of these questions.

Since neurones of the retina, LGd, SC and visual cortex are generated at different times and have different schedules and tempo of differentiation, it is essential to examine first the time of origin of neurones of each structure, mode of their migration, and pattern of their distribution at different stages of development. After that, the development of connections will be described.

2. Time of origin of neurons in the monkey visual system

Since the time of origin of neurons cannot be determined by direct examination of histological preparations, we have been using the method of [^{3}H]thymidine autoradiography to obtain data on the 'birthdays' of cells destined to form the visual system in monkey (Rakic 1974*a*, 1975*a*, 1976*a*, *b*). The 'birthday' of a neuron is empirically defined as the last day on which nuclear DNA is replicated in the progenitor of a given cell (Angevine 1965). Neurons can be classified as non-renewing, static epithelium (Leblond 1964) and each class of neurons has a precisely scheduled range of 'birthdays' (Sidman 1970). The autoradiographic procedure involves exposing embryonic brain cells to [^{3}H]thymidine by injecting pregnant monkeys with this DNA precursor at different stages of gestation. [^{3}H]thymidine crosses the placenta and becomes available to dividing cells of the monkey embryo for less than 10 min (Nowakowski & Rakic 1974). When the offspring of such pregnancies are sacrificed 2–5 months after birth, the progeny of cells which incorporated [^{3}H]thymidine into their genome during their last

Downloaded from rstb.royalsocietypublishing.org

round of DNA synthesis remain permanently radioactive and can be detected in autoradiograms as heavily labelled. For this study the time of neuron origin in the retina, LGd, SC and primary visual cortex has been examined in a series of 20 juvenile rhesus monkeys (*Macaca mulatta*) that had been exposed to a pulse of [^{3}H]thymidine (10 μCi/g) at various pre- and early postnatal ages (table 1). Details on experimental procedures can be found in Rakic (1973, 1974*a*).

Table 1. The time of origin of neurons in the visual system in the rhesus monkey as determined by [^{3}H]thymidine autoradiography

(Each monkey was exposed to a pulse of [^{3}H]thymidine at different embryonic (E) or postnatal (P) days and all animals were sacrificed between 2 and 5 postnatal months. Sign plus (+) indicates the presence of numerous heavily labelled neurons, whereas sign (±) indicates that only an occasional heavily labelled neuron has been found. Abbreviations: RGC, retinal ganglion cells; LGd, neurons in the dorsal lateral geniculate body; SCsg, neurons of the stratum griseum superficiale of the superior colliculus. Brackets in column 4 (area 17) designates times when neurons of layer IV, that receive direct projections from the LGd, are generated.)

specimen number	injected on embryonic (E) or postnatal (P) day	sacrificed at postnatal (P) day	1 RGC	2 LGd	3 SGsg	4 area 17
101673A	E30	P76	±	–	–	–
052274B	E33	P64	±	–	–	–
072673B	E36	P54	+	±	±	–
101774	E38	P72	+	+	+	–
031373	E40	P62	+	+	+	–
120574B	E43	P70	+	+	+	±
092371	E45	P58	+	–	+	+
051673	E50	P61	+	–	+	+
080872A	E54	P110	+	–	+	+
020370	E62	P50	+	–	–	+
080872B	E70	P98	±	–	–	[+
031870	E80	P48	±	–	–	+] IV
103073	E90	P63	–	–	–	+
072971	E102	P65	–	–	–	±
051575	E110	P62	–	–	–	–
101570	E120	P113	–	–	–	–
073070	E140	P57	–	–	–	–
012770	newborn	P150	–	–	–	–
100973A	P18	P147	–	–	–	–
101673B	P32	P96	–	–	–	–

(*a*) *Retinal ganglion cells*

A few heavily labelled retinal ganglion cells (RGC) are found in the eyes of a juvenile monkey that had been exposed to a pulse of [^{3}H]thymidine at embryonic (E) day E33 (figure 2*a*, *b*, plate 1). Occasionally labelled neurons are present also in an animal exposed to this DNA precursor at E30 but by far the greatest majority of retinal ganglion cells in rhesus monkey are generated during the one-month period between E33 and E62 (table 1). Some heavily labelled ganglion cells can be seen in an animal injected at E70; and only a few can be found at the far periphery of the retina after exposure to [^{3}H]thymidine even at later stages (La Vail & Rakic, in preparation).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

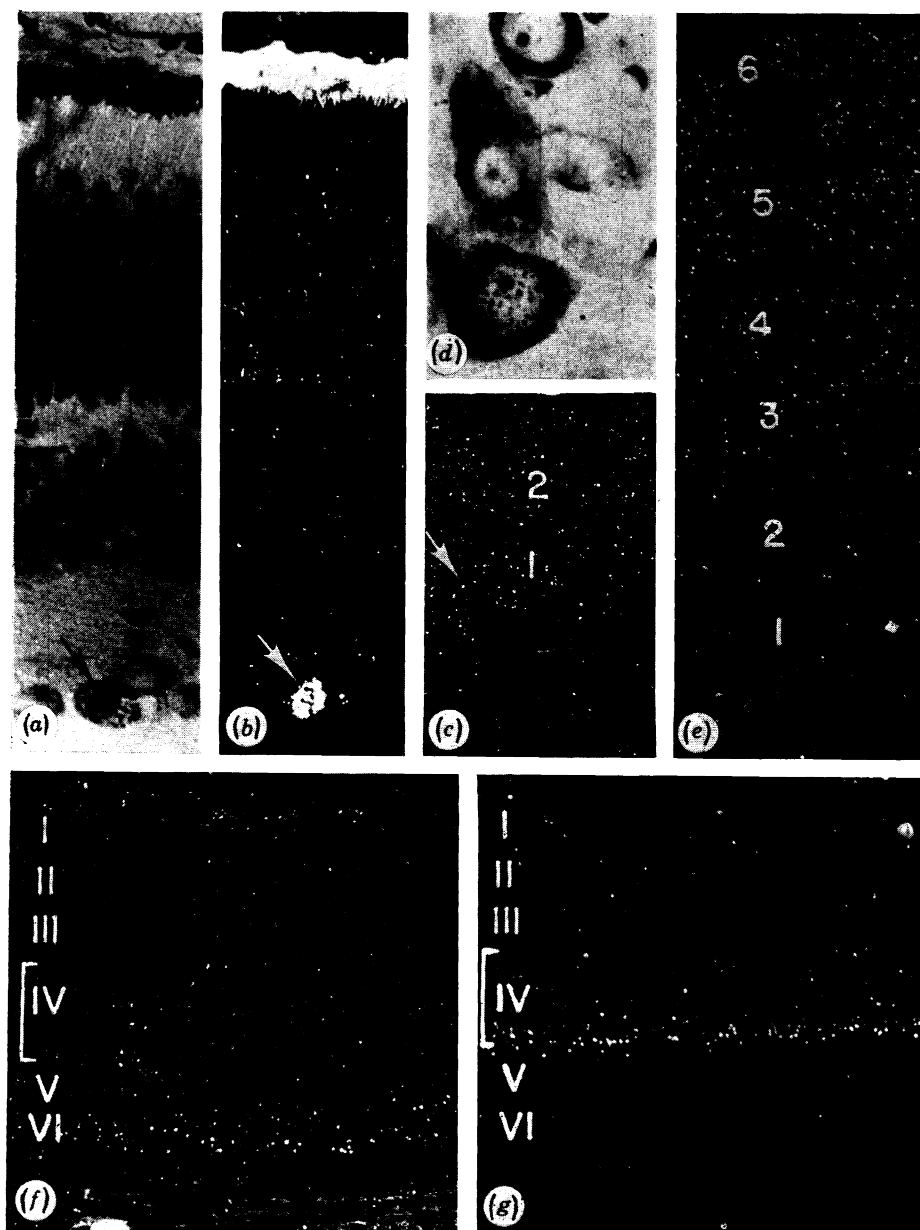


FIGURE 2. Bright- and dark-field photographs of labelled neurons in the visual system of two- to three-month old postnatal (P) monkeys that had been exposed to [H^3]thymidine at various embryonic (E) days. (a) Heavily labelled retinal ganglion cell (arrow) in E36–P54 animal. Bright-field photograph across the full thickness of the retina. (b) Similar cell photographed in dark-field illumination. (*c*) Low magnification dark-field photograph of the E38–P60 animal displaying labelled cells in magnocellular layers 1 and 2 of the LGd. (d) Bright-field photograph of the large labelled neuron marked in figure 2*c* by arrow. (e) Low power dark-field photograph across entire thickness of the lateral geniculate body in E43–P70 animal. Labelled neurons are predominantly located in layers 5 and 6. (*f*) Area 17 in the E62–P50 animal. Heavily labelled neurons are located in layer VI and deep zone of layer V. (*g*) Corresponding sector of area 17 in E70–P98 animal. Heavily labelled neurons are concentrated mostly in the deep zone of layer IVC.

(*Facing p.* 248)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

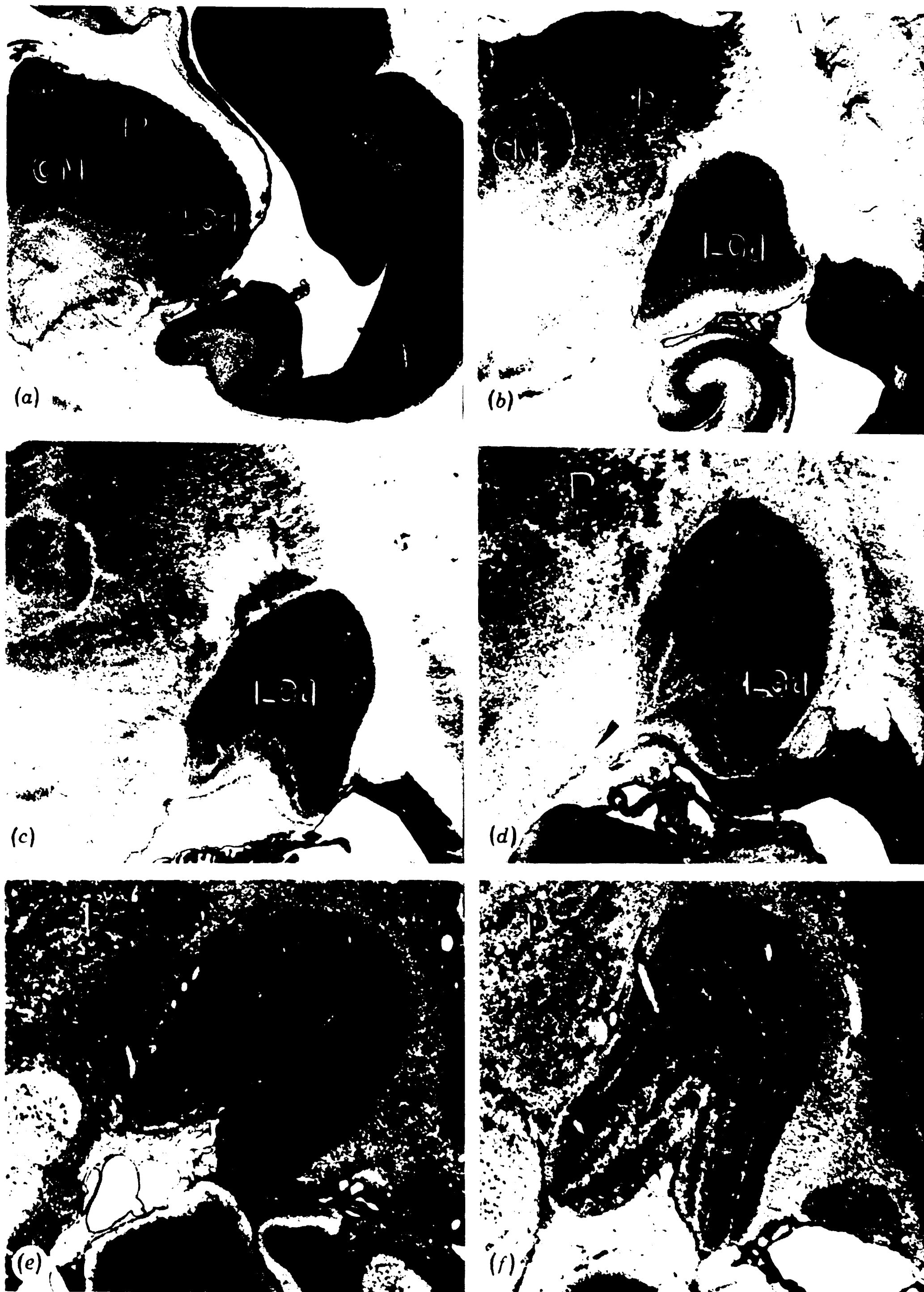


FIGURE 4. The position, shape and cell distribution of the dorsal lateral geniculate body in rhesus monkey diencephalon at different foetal and postnatal ages. Cresyl violet stained 35 μm thick sections, courtesy of Dr P. I. Yakovlev. The foetal ages were estimated by comparing these specimens with specimens cut at 8 μm in my collection of over 80 monkey foetuses in which pregnancies were precisely timed. Estimated age is approximately E70 (at *a*); E90 (at *b*); E110 (at *c*); E125 (at *d*). At *e* is a newborn and at *f* a 91-day-old monkey. Cell free, fibre layers that separate laminae are clearly indicated in older specimens, but are not present at E70 and E90, although numerous axons from both eyes have invaded LGd (see figure 5*b*). Further explanation in text. Abbreviations: CM, nucleus centre medanium; LGd, dorsal lateral geniculate body; P, pulvinar.

BIOLOGICAL SCIENCES THE ROYAL SOCIETY PHILOSOPHICAL TRANSACTIONS OF
BIOLOGICAL SCIENCES THE ROYAL SOCIETY PHILOSOPHICAL TRANSACTIONS OF

Downloaded from rstb.royalsocietypublishing.org

(*b*) *Dorsal lateral geniculate body*

Examination of the LGd in the autoradiograms from the same series of monkeys showed the presence of heavily labelled neurons in animals exposed to a pulse of [^{3}H]thymidine at E36, E38, E40 and E43, but not in monkeys exposed to this isotope at E33 or earlier or at E45 or later (table 1). Thus, the interval between the time of origin of the first retinal ganglion cells and the first neurons in the LGd is at least 3–6 days.

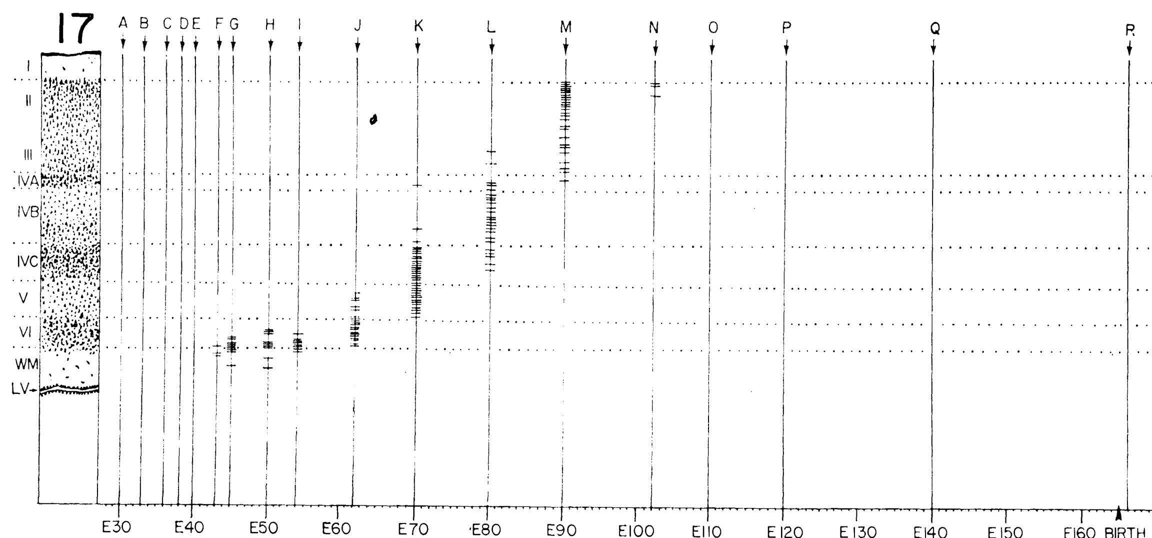


FIGURE 3. Diagrammatic representation of the positions of heavily labelled neurons in the primary visual cortex (area 17 of Brodmann in the depth of calcarine fissure) of juvenile monkeys, each of which had been injected once with [^{3}H]thymidine at selected embryonic days. The drawing of the cortex on the left side of the diagram is made from cresyl violet-stained sections. Divisions into cortical layers are indicated by Roman numerals according to the classification of Brodmann (1905). Embryonic days (E) are represented on the horizontal line, starting on the left with the end of the first foetal month (E28) and ending on the right at term (E165). Positions of vertical lines (A to R) indicate the embryonic day on which each animal received a pulse of [^{3}H]thymidine. Labelled neurons were found only in 9 animals. The heavily labelled cells in the cortical laminae are first located by examination in dark- and bright-field microscopy and then their exact position within the cortex was plotted by the use of a Zeiss microscope equipped with a drawing tube. The relative position of labelled cells within the cortex and their distance from the pia is marked by a short horizontal bar on the vertical line that traverses the entire width of the cortex. On each vertical line (except in line N), short horizontal markers indicate positions of all heavily labelled neurons encountered in one 2.5 mm long strip of the cortex. Since the number of labelled neurons decreases towards the end of neurogenesis, the three labelled neurons in area 17 indicated on the vertical line N (E102) were found only after examination of 80 areas of calcarine cortex each 2.5 mm wide in 40 autoradiograms. Abbreviations: LV, obliterated posterior horn of the lateral ventricle; WM, white matter (modified from Rakic 1974*a*).

In the LGd, neurons destined for the ventral magnocellular layers are generated earlier than those destined for the dorsal parvocellular layers (Rakic 1976*b*). Thus, in juvenile monkeys exposed to [^{3}H]thymidine at E38, heavily labelled cells are situated predominantly in layers 1 and 2 (figure 2*c*, *d*) although a few are found also in layer 3 (figure 2*c*), whereas in animals injected at E43, labelled cells are present mostly in layers 5 and 6 (figure 2*e*). However, the correlation between the time of origin of neurons and their eventual laminar distribution in the adult is less pronounced in the LGd than in the monkey visual cortex (see below and Rakic 1974*a*).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(c) *Superior colliculus*

With respect to the superior colliculus, I report here only the time of origin of neurons which receive direct input from the two eyes, i.e. those of the stratum griseum superficiale (SCsg). Genesis of SCsg neurons begins at about the same time (E36) that LGd neurones are first generated (table 1). However, the total time during which neurons of the SCsg are produced is longer than in LGd and small numbers of labelled neurons have been observed even in animals that had been injected at E54. In the SCsg, the neurons situated in deeper positions tend to be generated earlier and those situated superficially at later periods.

(d) *Primary visual cortex*

In the primary visual cortex (area 17 of Brodmann 1905), heavily labelled neurons were found in juvenile animals that had been exposed to a pulse of [^{3}H]thymidine at E43, E45, E50, E54, E62, E70, E80, E90 and E102 (table 1). No labelled neurons were found in animals that had been injected either before E43 or after E102. Therefore, in the rhesus monkey the production of neurons destined for the visual cortex takes place over about a two-month period in the middle of gestation and is completed approximately two months before birth.

The final position of neurons in the cortex correlates systematically with the time of cell origin; neurons destined for positions in deeper cortical layers are generated earlier and those situated more superficially progressively later (figure 2*f*, *g* and figure 3). From inspection of the maps in figure 3, it is evident that most of the neurons destined for layer IV, which receive direct input from the LGd, are generated between E70 and E85. The last generated neurons are found at the borderline between layers I and II. In general, the migration pattern of neurons of the monkey visual cortex corroborate the 'inside out' gradient of cell disposition described in rodents (Angevine & Sidman 1961; Berry & Rogers 1965; Hicks & D'Amato 1968; Shimada & Langman 1970), but in the primate, this gradient is more pronounced; i.e. neurons generated simultaneously are confined to a narrower stratum of the cortex than they are in rodents (Rakic 1974*a*). Correlation of human and monkey cortices indicate that in man the full complement of neurons destined for the visual cortex is also generated well before birth (Rakic 1975*a*).

3. Migration and secondary redistribution of neurons following their last cell division

Autoradiographic analysis of another series of monkeys exposed to pulse of [^{3}H]thymidine at various embryonic ages and sacrificed at short intervals indicates that neurones are not generated in visual centres but rather that following their last cell division in the proliferative zones at the ventricular surface, young neurons migrate varying distances to the site where they will be incorporated into synaptic circuits. Furthermore, since the formation of visual connections occurs at developmental periods when visual centres are not as yet fully differentiated, it is essential to determine accurately the position and distribution of neurons in these structures during various ontogenetic stages. As part of an ongoing project on the development of the primate visual system, I have so far analysed only the histogenesis of the LGd (Rakic 1976*b*, 1977) and the visual cortex (Rakic 1974*a*, 1975*a*, 1976*a*).

Downloaded from rstb.royalsocietypublishing.org

(*a*) *Dorsal lateral geniculate body*

Examination of embryos killed 1 h, 3 days or 7 days after exposure to [^{3}H]thymidine shows that cells destined for the LGd proliferate at the surface of the third ventricle. Following their last cell division, young neurons migrate to the lateral aspect of the developing diencephalon; the first generated neurons reach the outer surface, those generated later stop at progressively deeper positions, i.e. in an 'outside-in' pattern as they do in the thalamus of the mouse (Angevine 1970). Thus, the distribution of young neurons in relation to their 'birthday' in the LGd is the reverse of the 'inside-out' pattern found in the mammalian cerebral cortex (see § 2*d*, above).

It should be kept in mind that at these early stages the LGd of the monkey is situated at the lateral aspect of the thalamus (figure 4, plate 2) as it is in adult rodents (Clark 1932) and in human foetuses during the first trimester (Rakic 1974*b*). Subsequently, due to the expansive growth of the pulvinar, the LGd is simultaneously rotated upon its transverse and sagittal axes and becomes displaced to the inferior aspect of the mature thalamus. As a consequence, neurons initially situated at the lateral surface of the diencephalon come to lie in a ventral position and constitute deep laminae of the LGd whereas the last generated cells become part of the dorsal laminae (Rakic 1976*b*, 1977).

Between E38, when the first neurons of the LGd arise, and the middle of gestation (E83) there is no visible aggregation of LGd neurons into a laminar pattern (figure 4*a*, *b*). Primordial laminae appear only during the last third of gestation and are discernible, although not completed, between E91 and E100 (figure 4*c*). Thereafter, the laminar pattern of the LGd becomes gradually clearer (figure 4*d*) and, by the time of birth, the laminar organization has assumed essentially the adult form (figure 4*f*). It is noteworthy that in man lamination of the LGd emerges at the 6th foetal month; i.e. at the beginning of the last trimester (Preobrazhenskaya 1965).

(*b*) *Primary visual cortex*

Studies of the histogenesis of primate visual cortex have shown that all young neurons are produced in the proliferative zones situated near the surface of the lateral cerebral ventricle and they subsequently migrate radially to the developing cortical plate (Rakic 1974*a*, 1975*a*). Since each generation of migrating neurons reaches the most superficial stratum of the developing cortical plate, their position in the adult cortex depends on the number of subsequently produced cells that eventually become situated external to them (Rakic 1975*a*). Therefore, as mentioned above, the 'inside-out' pattern of cell position in relation to the time of their origin is reversed from that which characterizes the initial formation of the immature LGd. As the cerebral wall expands, post-mitotic young neurons traverse increasingly longer distances to reach the superficial strata of the developing cortical plate. The mechanism of cell migration to the primate cortex is described and discussed elsewhere (Rakic 1972, 1975*a*).

The timetable of 'birthdays' of neurons that compose each cortical layer in the region of area 17 situated in the depth of the calcarine fissure is given in figure 3, and this subject is more fully elaborated in Rakic (1975*a*). Precise timing of the genesis of neurons of each cortical lamina in relation to the arrival of afferents may be essential for the establishment of laminated structure and for the formation of neuronal connections. The failure of migrating cells to attain their proper position according to a specified time schedule may severely compromise the orderly development of the laminar pattern in the cortex (Rakic 1975*b*).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

4. Pattern of distribution of the visual connections at various foetal ages

The mode of development of visual connections was studied autoradiographically (Cowan *et al.* 1972; Wiesel *et al.* 1974; Rakic 1976*d*). Equal amounts of [^{3}H]proline and [^{3}H]fucose (total 1.0–1.5 mCi) were injected into the vitreous body of one eye of each of three monkey foetuses at different gestational ages. Following this injection, the foetuses were returned back into the uterus for 14 days (table 2). The survival period of 14 days was chosen to allow transneuronal transport of the radioactive label to second order neurons and their axons. The surgical procedure and methods of preventing abortion following injection and retrieving the foetus will be described elsewhere.

Table 2. Summary of experimental procedure for the three foetal monkeys injected once each at specific embryonic (E) day into one eye with equal parts of [^{3}H]proline and [^{3}H]fucose

Following injections, each foetus was replaced into the uterus and sacrificed 14 days later.

animal	embryonic day (E) when injected	embryonic day (E) when sacrificed	exposure time for LGd and SCsgs (in weeks)	exposure time for cortex (in weeks)
1 (052775)	E64	E78	2, 4	2, 4, 8, 16, 20
2 (021075)	E110	E124	2, 4	2, 4, 8, 16, 20
3 (061275)	E130	E144	2, 4	2, 4, 8, 16, 20

(*a*) *Retinogeniculate projection*

The youngest foetus in this series was injected at E64 because, according to our [^{3}H]thymidine data, all LGd neurons have been generated before this age (table 1) and the foetus was sacrificed at E78 when the LGd has not yet become laminated (see §3*a* above, and figure 4).

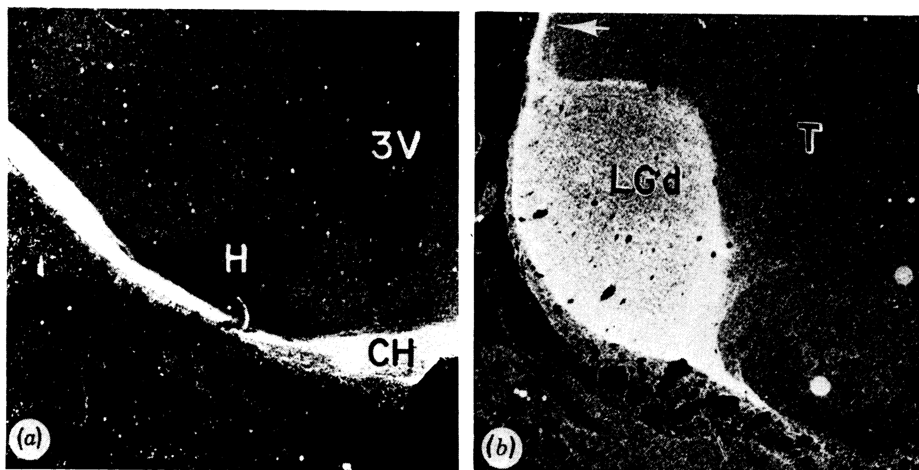
Description of plate 3

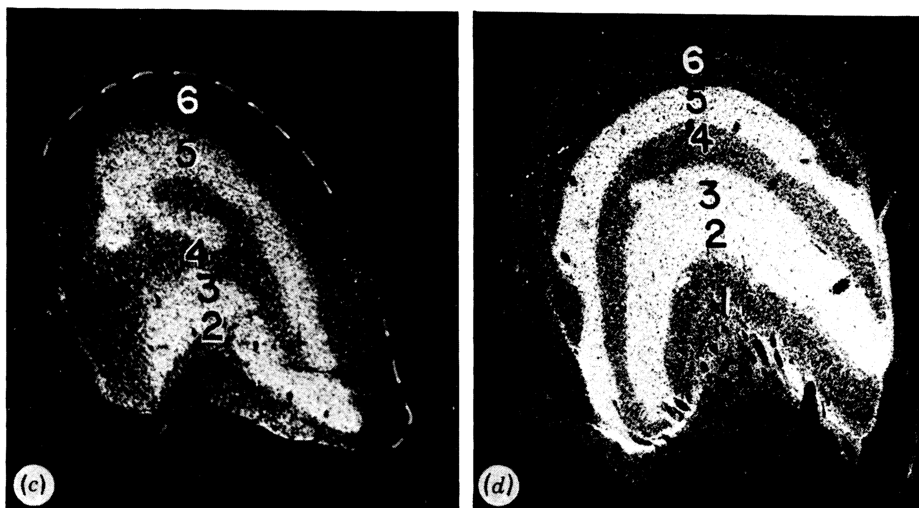
Figure 5. Dark-field photographs of autoradiograms from foetuses removed from the uterus and injected in the vitreous body of one eye with a mixture of [^{3}H]proline and [^{3}H]fucose at different embryonic (E) days. Foetuses were replaced in the uterus and sacrificed 14 days later by the second caesarian section. All photographs are taken at the same magnification (×20). (*a*) Optic chiasm (CH) and left optic tract in the foetus injected at E64 and sacrificed at E78 are heavily labelled. (*b*) Contralateral LGd in the same foetus (E64–E78). Radioactive label does not display a laminar pattern. The white arrow indicates the brachium of the superior colliculus. (*c*) Ipsilateral LGd in the foetus injected with comparable doses of the same isotope at E110, sacrificed at E124. Label is concentrated over incipient laminae 2, 3 and 5. (*d*) Ipsilateral LGd in the foetus injected at E130 and sacrificed at E144. Label is concentrated over the more regularly shaped and sharply defined layers 2, 3 and 5. Abbreviations: H, hypothalamus; LGd, dorsal lateral geniculate body; 3V, third ventricle; arabic numerals denote layers in the LGd.

Figure 6. (*a*) Dark-field photograph of a coronal section of the contralateral superior colliculus (SC) of the foetus which had one eye injected *in utero* at E64 with a mixture of [^{3}H]fucose and [^{3}H]proline and was sacrificed at E78. Label is relatively uniform over the entire nucleus. (*b*) Bright-field photograph of a sagittal section of the contralateral SC in the foetus that had been injected with the same mixture of radioactive labels at E110, replaced *in utero* and sacrificed at E124. Retinotectal projections are clearly segregated into ocular dominance patches indicated by areas of low grain counts. (*c*) Bright-field photograph of a somewhat more medial level of the SC from the same specimen shown in *b*. The large empty zone corresponds to the optic disk (OD) representation. (*d*) Bright-field photograph of a transverse section of the ipsilateral SC in the foetus injected and killed at slightly later gestational ages (E130–E144). Retinotectal projections are concentrated into discrete patches. (Magn. × *ca.* 20.)

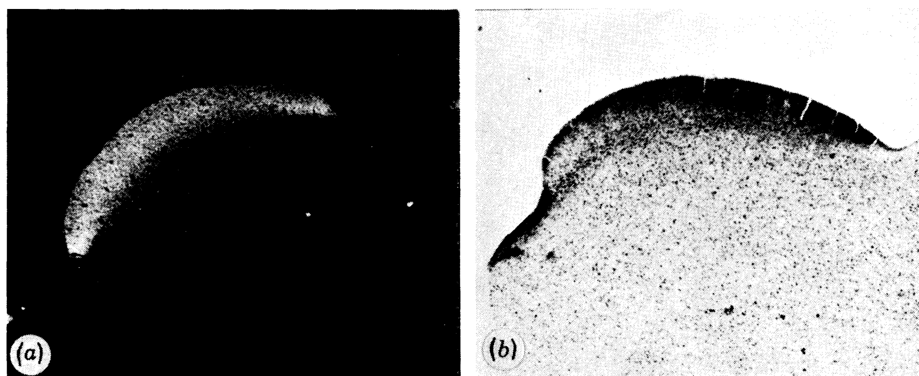
Downloaded from rstb.royalsocietypublishing.org

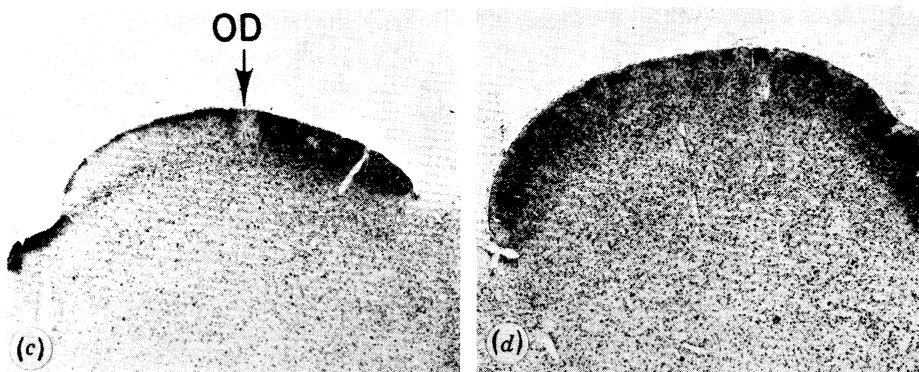
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES





5





6

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURE 5 AND 6. For description see opposite. *(Facing p.* 252)

Downloaded from rstb.royalsocietypublishing.org

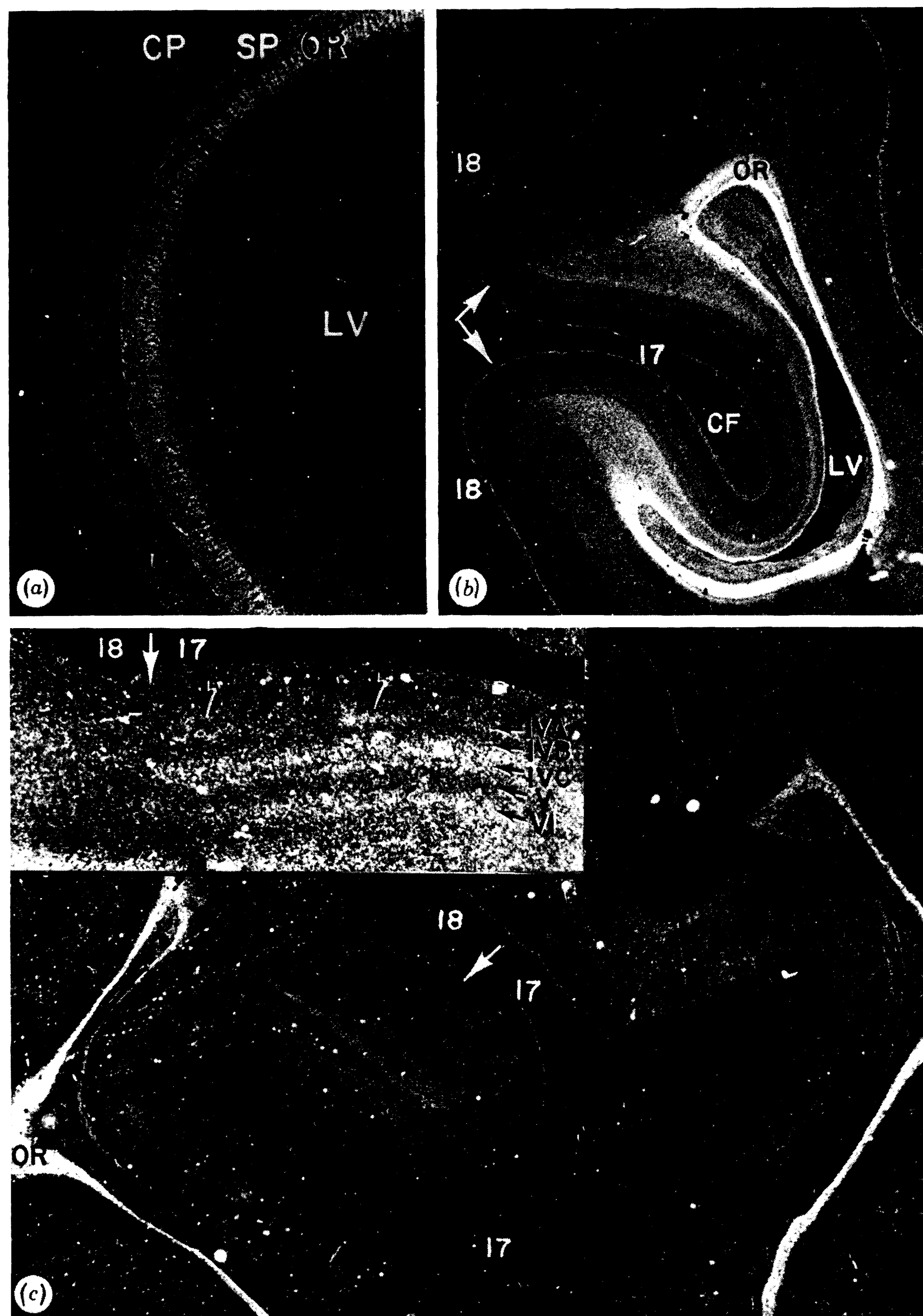


FIGURE 7. (*a*) Dark-field photograph of the occipital lobe of the fetus injected with a mixture of [^{3}H]proline and [^{3}H]fucose at E64, sacrificed at E78. The large lateral ventricle (LV) is on the right. Optic radiation (OR) is heavily labelled by transneuronal transport of isotopes, but fibres stop at the subplate layer (SP) and do not invade the developing cortical plate (CP). (*b*) Occipital lobe of the foetus whose one eye was injected with the same mixture of labels at E110 and sacrificed at E124. The optic radiation surrounds the lateral ventricle (LV) and emanates axons only to area 17 of the calcarine fissure (CF). Projections to the cortex stop sharply at the borderline of area 18 (arrows). Some axons and/or terminals have entered the cortical plate, but are distributed uniformly over layer IV. (*c*) Visual cortex of the foetus injected and sacrificed at a slightly older age (E130–E144). More axons and/or terminals have invaded the cortex and are concentrated over layer V and VI. Segregation of axons and/or terminals over sublayers IVA and IVC, as well as alternating stripes of higher (black arrowheads) and lower grain counts are barely indicated in the dark-field inset of an autoradiogram exposed 16 weeks. For further details, see text and Rakic (1976*d*).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

This experiment was designed to determine whether axons from each eye are segregated in the LGd before it develops laminae.

The radioactivity was transported in an orthograde manner from the injected eye to the optic nerve, through the optic chiasm and the optic tracts and into both LGds (figure 5*a*, *b*, plate 3). The LGd in each hemisphere displays approximately equal levels of radioactivity, indicating that both crossed and uncrossed retinal fibres are present in both nuclei at this age. Although there are areas with slightly higher and lower grain counts, fibres and/or terminals from each eye do not segregate into a layered pattern that even remotely resembles the pattern of the adult. These findings indicate that at early stages of development projections from both eyes overlap in their distribution among neurones of the LGd and that a separation into discrete compartments occurs secondarily (Rakic 1976*d*).

The second foetus in this series was injected in one eye with a mixture of [^{3}H]proline and [^{3}H-]fucose at E110 after the LGd already shows incipient lamination (figure 4*c*). The foetus was sacrificed fourteen days later at E124. Although the laminae in the LGd at this stage are still rather irregular in shape, one can easily distinguish a pattern of 6 layers. The primordial laminae 2, 3 and 5 are radioactive in the LGd on the side of the injected eye (figure 5*c*) and 1, 4 and 6 are radioactive on the opposite side (Rakic 1976*d*). Therefore, the segregation of input from two eyes occurs sometime between E64 and E110.

The distribution of the radioactive label in the third foetus that was injected and killed at slightly later gestational ages (E130–E144) was essentially the same as in the previous foetus, except that the laminar organization of the LGd was more regular and more sharply outlined, resembling closely the adult pattern. Only the three appropriate laminae of the LGd on each side were radioactive (figure 5*d*). High power dark-field microscopy shows that the radioactive label is distributed mostly in the developing neuropile over areas between neurone somas.

(*b*) *Retinotectal projections*

A relatively large amount of radioactivity is transported through the brachium of the superior colliculus (arrow in figure 5*b*) into the tectum in the foetus injected at E64. Although present in both SC at the time of sacrifice at E78, the radioactivity is somewhat more dense in the contralateral side. The distribution of label in the SC on both sides is relatively uniform (figure 6*a*, plate 3) and does not show the characteristic patchy distribution of ocular dominance areas described in the adult monkey by Hubel *et al.* (1975).

The distribution of radioactive grains over the tectum in the foetus injected at E110 and killed at E124 is significantly different from that in the youngest foetus. The grains situated over the SC now form areas of heavy and light concentrations (figure 6*b*). Although not defined as sharply and clearly as in adult monkeys, these areas exhibit an alternating pattern (figure 6*b*) that closely corresponds in number and pattern the ocular dominance 'clumps' described in the adult (Hubel *et al.* 1975). The patchy pattern is most pronounced in the posterior portion of the SC where projections enter more deeply into the superficial gray. The dimension of the patches that contain high and low concentrations of grains is variable but, in general, ranges from 0.07 to 0.3 mm in width. The smaller size of these patches compared to those described in adult monkeys by Hubel *et al.* (1975) is probably due to subsequent growth of the SC. The absence of a projection from the contralateral eye to the region of the optic disk representation at this foetal age is striking and is similar to the situation in postnatal monkeys (OD in figure 6*c*).

The oldest foetus in the series of experiments injected with the proline-fucose mixture at

Downloaded from rstb.royalsocietypublishing.org

E130 and killed at E144 shows essentially the same pattern of grain distribution as that found in the foetus injected at E110 except that ocular dominance clumps are somewhat larger and more clearly defined (figure 6*d*).

(*c*) *Geniculocortical projections*

In all three foetuses that received injections of the [^{3}H]proline and [^{3}H]fucose mixture into one eye, radioactive label was transported transneuronally from RGC axons to the principal neurons of the LGd and then moved orthogradely in geniculocortical axons as revealed by a high concentration of label over the optic radiations (figure 7*a–c*, plate 4). Since the amount of radioactivity transported transneuronally seems to be less than 3% of the amount present in the primary pathway (Grafstein 1971) the exposure time for autoradiograms has to be several times longer to demonstrate its distribution adequately (table 2). The high background in these autoradiograms is due to unspecific labelling and is present also over autoradiograms of the liver, kidney, spleen and other organs prepared from the same foetus. Therefore, only a concentration of grains well above background can be considered to represent radioactivity in the axons of the visual pathways.

In the youngest foetus injected at E64 and sacrificed at E78, fibres from the optic radiation (OR in figure 7*a*) have reached the occipital lobe, but they do not seem to enter the developing cortical plate in any substantial number. Rather, most of the radioactivity is concentrated below the cortical plate in the intermediate zone of the developing cerebral wall where the grains form a continuous and sharply outlined band situated external to the optic radiation (SP in figure 7*a*). The distribution of grains is rather uniform, without any periodicity and hence, at this stage ocular dominance columns could not be demonstrated. This should be expected, since in this foetus, fibres from both eyes are not as yet segregated at the level of the LGd (see §4*a* above).

It should be emphasized that at the age when the youngest foetus was sacrificed (E78), only a fraction of the cortical neurons destined for layer IV have been generated (figure 2*d*). Furthermore, due to the variable speed of cell migration in the monkey telencephalon at this foetal age (Rakic 1975*a*), many neurons which have had their last cell division are still located in the intermediate zone as they migrate to the cortex. These migrating neurons are passing through the region occupied by the geniculocortical fibres. The relationship of young neurons situated temporarily in the intermediate zone to the afferents from LGd needs to be analysed by EM autoradiography to determine if there are any specialized types of membrane junctions between LGd axons and these migrating cortical cells at this age.

The second foetus was both injected (E110) and killed (E124) after all cortical neurons are generated and after they have reached their position in the cortex (Rakic 1975*a*). LGd axons are well distinguished by a dense concentration of grains over the optic radiation (OR) in the occipital lobe (figure 7*b*). Fibres emanating from the optic radiation towards the cortex stop sharply at the borderline of area 17 (figure 7*b*). Even at this relatively late foetal age, large numbers of fibres fail to invade the cortical plate itself as indicated by a dense concentration of grains in the zone below the primary visual area. However, detectable numbers of fibres have entered the cortical plate and are demonstrable by the uniformly distributed grains over layers VI and IV (figure 7*b*). The distribution of grains over layer IV does not show separation into sublayers 4A and 4C or alternating periodicity of the ocular dominance columns.

In the oldest foetus (E130–E144), more geniculocortical fibres have settled in the cortical

Downloaded from rstb.royalsocietypublishing.org

plate, as indicated by a somewhat greater amount of radioactive label present over layers VI and IV. In this specimen, an indication of a bilaminate pattern with concentration of silver grains over sublayers IVA and IVC can be discerned in autoradiograms exposed 12–16 weeks (figure 7*c*), but boundaries between these two sublayers are not as sharply delineated as in the adult. Although grains over sublayers IVA and IVC form a continous sheet, sectors with slightly higher grain concentration that alternate with sectors with lower grain concentration begin to emerge. This uneven distribution of grains is barely visible in dark-field microphotographs but counts of grains indicate clearly the presence of such areas (Rakic 1976*d*). The distribution of label into areas of higher and lower grain counts occurs in parallel over both sublayers and indicates the beginning of a separation of the input from each eye. Thus, although ocular dominance columns are not completely developed, a hint of their pattern is indicated three weeks before birth.

5. Discussion

(*a*) *Neuronal migration and redistribution in visual centres*

Neuronal elements and their processes undergo considerable changes in position during prenatal genesis of the visual system in the rhesus monkey. These cellular activities are well coordinated in space and in time to allow formation of complex cytoarchitectonic patterns and point to point connectivity of widely separated structures. However, spatial changes that occur during embryogenesis probably vary in their significance for the development of connections.

The initial redistribution of young neurons occurs following their last cell division when post-mitotic neurons migrate from the site of their origin near the ventricular surface to positions in cortical or subcortical structures where they will eventually become incorporated into synaptic circuits. This phenomenon is a generalized characteristic of the developing central nervous system (Sidman & Rakic 1973) and has been observed in all subdivisions of the visual system in the foetal monkey brain. The specific significance of migration in the formation of normal neuronal connections is not clear although failure of migration can severely alter synaptic circuitry (Rakic 1976*c*). For example, we do not know whether neurons generated at a given time and migrating to specific destinations are already programmed to differentiate into a specific class of neurons or, alternatively, whether migrating neurons are 'indifferent' cells that will be specified by interaction with the local cellular milieu. [^{3}H]thymidine autoradiography tells us only that there is an orderliness and predictable correlation between the time of neuron origin and the final positions which neurons assume in the adult central nervous system. This correlation is particularly clear in the primate visual system.

After neuronal migration is completed and young neurons have arrived at a given structure, a secondary displacement of neuron somas and development of their processes occurs as an interim step in the genesis of the adult structural pattern. In the SC and primary visual cortex, this type of cellular redistribution may be less apparent but in the LGd, either neurons alone, afferents alone, or neurons already conjugated with afferents change their position considerably to form connections and the laminated organization of the adult. Shifting of post-migratory neuronal cell bodies may merely represent a consequence of the development of neuropile. Whether the neurons of the LGd or the fibres from the retina play a principal role in the process of lamination cannot be answered conclusively from the presently available material. A series of experiments that involve enucleation of one or both eyes at different

Downloaded from rstb.royalsocietypublishing.org

BIOLOGICAL SCIENCES THE ROYAL SOCIETY PHILOSOPHICAL TRANSACTIONS OF

embryonic ages is underway in my laboratory and will hopefully shed some light on this problem.

As the cells of the LGd become redistributed, the entire nucleus is passively shifted to a ventral position in the thalamus, as discussed in Rakic (1977) and in §3*a*. This displacement, although important for understanding the changes in relative position of different parts of the LGd in ontogeny, and for the interpretation of findings on axonal ingrowth to the LGd, it may be of little consequence for specificity in the formation of visual connections.

(*b*) *Ingrowth of afferents into developing visual centres*

An important consideration in understanding the genesis of visual connections is the relationship between the timing of migration of post-mitotic cells to their final locations in any given visual centre and the timing of arrival of afferents with which they eventually form synaptic contacts. Theoretically, afferents might arrive at the appropriate position before neurons of a given structure are generated, or they might join migrating neurons on their way to their destinations, or they might enter target structures after neurons have already attained their positions. Unfortunately, the methodology presently available is inadequate to determine the time of formation of the earliest connections with precision. For example, the time of arrival of the very first axons from the retina to the LGd and SCsg is still not known. The extirpation of an eye or the injecton of radioactive amino acids in the eye at such early stages of monkey ontogenesis (between E33 and E36) is impractical if not impossible. However, [^{3}H]thymidine autoradiographic data show that a time lapse of several days between the genesis of the first ganglion cells and the first neurons of the LGd and SCsg (table 1) allows for the possibility that pioneer axons from the retina might arrive at the diencephalon and midbrain before neurons destined for the LGd and SC are generated or while they are still in the migratory phase.

The data on ingrowth of the geniculocortical fibres are, in this respect, somewhat more detailed since the cortex develops at comparatively later foetal ages. Thus, LGd neurons form the optic radiation before E78, but axonal endings initially do not invade the cortical plate; instead axons accumulate in the intermediate zone below the developing cortical plate. At this foetal age, the majority of neurons of layer IV, which will eventually receive input from geniculocortical axons are not as yet generated and many of those that have been generated are in the process of migration. Our observations indicate that geniculocortical axons remain below the cortex and 'wait' there in a densely packed fibre layer until the conditions become established for their entry into the cortical plate. Therefore, LGd fibres may contact migratory neurons passing through the intermediate zone, before they reach the appropriate level in the cortex.

Formation of a transient layer below the cortical mantle by axons from the LGd is not a unique example of such phenomena in the developing central nervous system. For instance, commissural fibres in the neonatal rat, after entering the contralateral hemisphere, remain in the white matter for several days before invading the cortex (Wise & Jones 1976). Another relevant example occurs during histogenesis of the human cerebellum, where a transient embryonic layer, the so-called *lamina dissecans* of Jakob (1928) develops between the Purkinje cell layer and the granular layer. This transient layer in the human foetus contains various cerebellar afferents including mossy terminals and a small number of immature synapses (Rakic & Sidman 1970; Zecevic & Rakic 1976). This lamina may represent a 'waiting compartment' of cerebellar afferents which becomes depleted as young granule cells 'pick up' their synapses while migrating through this layer. A somewhat similar situation occurs during development

BIOLOGICAL SCIENCES THE ROYAL SOCIETY PHILOSOPHICAL TRANSACTIONS OF

Downloaded from rstb.royalsocietypublishing.org

of the optic tectum in the rat (Lund & Bunt 1976). Initially, synapses are formed at the surface of the SC, whereas in adult rat the synapses are situated in the stratum opticum, deep to the neurons in the stratum griseum superficiale. Either the synapses sink in from the surface, or neuronal somata migrate secondarily through the synaptic zone to the surface and displace the synapses ventrally in a manner similar to the penetration of the lamina dissecans by cerebellar granule cells. It is important to note that in the human foetal telencephalon synapses have been observed in the intermediate zone below the cortical layer in the so-called *subplate* layer (Kostovic & Molliver 1974). The origin of axons making these synapses in man is still not known. However, the subplate layer is located at approximately the same level of the intermediate zone where thalamic fibres accumulate in our monkey autoradiograms.

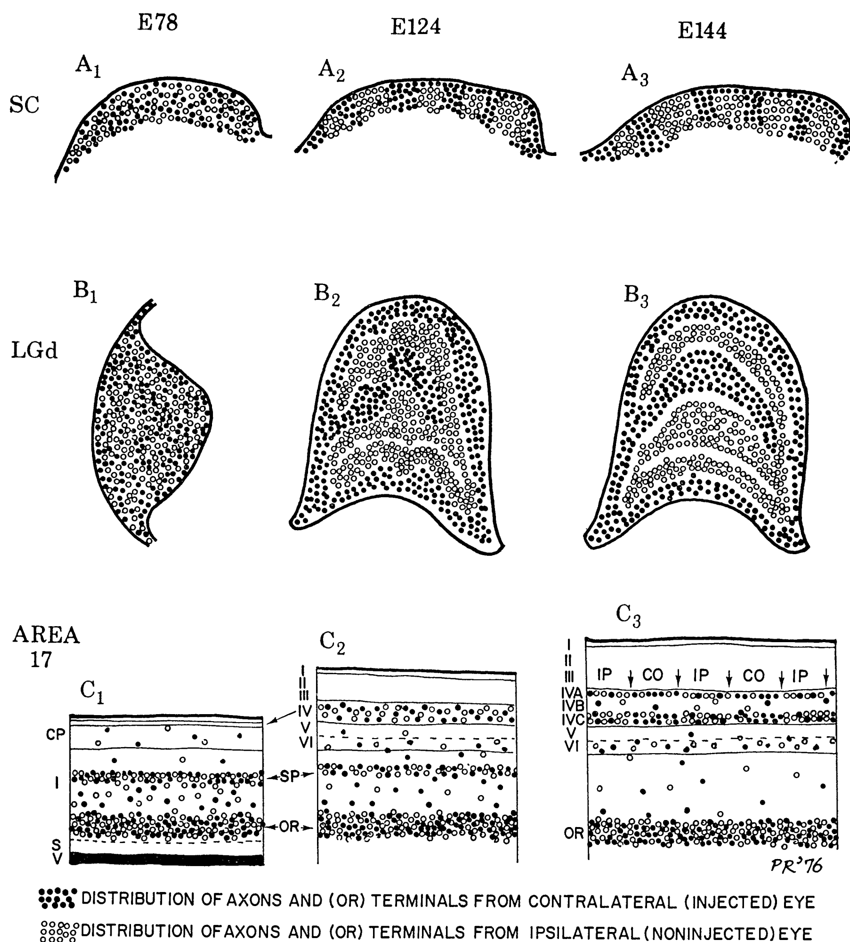


FIGURE 8. Schematic representation of the pattern of distribution of axons and/or terminals from the contralateral (filled circles) and ipsilateral (open circles) eye in the superior colliculus (SC, A_1, A_2, A_3) dorsal lateral geniculate body (LGd, B_1, B_2, B_3) and transneuronally through LGd axons in area 17 of the visual cortex (C_1, C_2, C_3) during prenatal development of the rhesus monkey. Diagram summarizes autoradiographic data obtained in one hemisphere after injecting a mixture of [^{3}H]proline and [^{3}H]fucose into the vitreous body of the contralateral eye in macaque foetuses 14 days before sacrifice at embryonic (E) day 78 – first column (A_1, B_1, C_1); at E124 – second column (A_2, B_2, C_2); and at E144 – third column (A_3, B_3, C_3). As indicated by the pattern of distribution of open and filled circles, inputs from both eyes initially overlap and subsequently segregate according to specific schedules in each structure. Further explanation in text. Abbreviations: CO, area with higher concentration of contralateral input; CP, cortical plate; I, intermediate zone; IP, area with higher concentration of ipsilateral input; OR, optic radiation; S, subventricular zone; SP, subplate; V, ventricular zone. Roman numerals indicate cortical layers. Note that at E78 only deep layers are present in the cortex.

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(c) *Development of binocular pattern*

Postnatal development of ocular dominance has been a topic of major interest since it was shown more than a decade ago (Wiesel & Hubel 1963) that neuronal function can be altered by monocular deprivation (for recent review, see Barlow 1975). We have focused on the structural development of the binocular systems before birth to determine to what extent these connections are specified before visual experience. Our data from prenatal injections of one eye with a [^{3}H]proline-fucose mixture indicate that initially input from one eye overlaps with that of the other eye in the LGd, SC and striate cortex but that before birth segregation into separate monocular areas either is completed as in the LGd and SC or has begun as in the striate cortex. The sequence of these events inferred from these experiments is schematically summarized in figure 8.

In the SC, projections from both eyes are first uniformly distributed throughout the full extent of the nucleus (A_1 in figure 8). By mid-gestation, input from a given eye is segregated in the form of monocular patches (A_2 in figure 8). In the cat, in which birth occurs at a comparatively earlier stage of brain development, the binocular organization of the SC is well-established more than 9 days before term (Graybiel & Nauta 1976).

In the LGd, projections from both eyes are also initially distributed in an overlapping manner (B_1 in figure 8). Our autoradiographic material indicates that segregation of input from each eye occurs between E78 and E124, concomitantly with the formation of a laminar pattern (B_2 in figure 8). Our present data do not allow us to resolve the question of whether fibres follow the displacement of neurons or neurons follow segregating axons. However, it is clear that binocular separation is attained well before birth in LGd (B_3 in figure 8).

Similar changes also occur in the visual cortex. Before (C_1 in figure 8), or immediately after afferent fibres from the LGd enter the cortex (C_2 in figure 8), they are uniformly distributed. After arriving at the cortex, afferent fibres have to segregate in two directions: vertically into the two strata of layer IV and horizontally to form alternating ocular dominance columns. In the oldest foetus (E130–E144) investigated in the present series of experiments, LGd axons in layer IV have become separated vertically into sublayers IVA and IVC. Ocular dominance columns are only barely visible at this foetal age although grain counts (Rakic 1976*d*) confirm their existence (C_3 in figure 8.) Monocular segregation into the columnar pattern is not completely developed even 7 days after birth (Hubel & Wiesel, personal communication). Therefore, the bilaminar organization of the cortex in macaque is established 3 weeks before birth while the monocular separation is only indicated at that time.

From our results, it is not clear whether or not the overlapping endings of axons from each eye in visual centres initially contact and/or synapse on the same neurons and subsequently become redistributed to two different cell populations, or, alteratively, axons remain 'uncommitted' until appropriate signals emerge. For example, some synapses are already present in the monkey LGd at E65 (Hendrickson & Rakic, in preparation) before the LGd is fully laminated. To distinguish between the two alternatives at this or any other level of the visual system, electron microscopic autoradiography has to be employed. In other systems, there are examples where two types of afferents overmingle or are situated in the reverse position during developmental periods before sorting out into their adult pattern (e.g. the climbing fibre and basket cell input to the cerebellar Purkinje cell (Larramendi 1969) or the olfactory bulb and association fibres terminals to neurons of the pyriform cortex (Price & Schwab 1976)). In these

Downloaded from rstb.royalsocietypublishing.org

instances, however, apparently rearranged terminals remain on the same neurons. Another relevant example is the development of the neuromuscular junction in rat where both anatomical and physiological evidence indicates that straited muscle cells at embryonic ages have multiple inputs all but one of which disappear around birth (Bennett & Pettigrew 1974). Our results indicate that in the monkey visual system connections are made in two steps: first, axons seem to be instructed to enter appropriate structures; and then secondly, their precise topographical distribution occurs.

Our data on the prenatal development of the visual system in the rhesus monkey demonstrate that the genesis of neurons comprising these structures as well as the basic connections between them including binocular organization is established before birth and are therefore specified before visual experience. Since primordial ocular dominance columns appear more than three weeks before birth, there is no reason to assume that their development is not governed by the same basic mechanisms involved in the formation of subcortical visual centres. The capacity for structural plasticity in the primate visual cortex expressed in response to various external agents and to the manipulation of the visual environment shortly after birth may utilize a vestige of the neuronal modifiabilty that is a basic property of the developing brain.

This work was supported by grant NS 11233 from the National Institutes of Health, U.S.A. I am thankful to New England Regional Primate Research Center, Southbourough, Massachusetts, for making timed pregnancies and surgical facilities available. Caesarian sections by Dr P. K. Sehgal as well as expert advice for technical improvements in intra-uterine surgery by Dr B. T. Jackson, Department of Obstetrics, Boston University Medical School, are gratefully acknowledged.

References (Rakic)

Angevine, J. B., Jr 1965 Time of neuron origin in the hippocampal region. An autoradiographic study in the mouse. *Exp. Neurol.*, Suppl. **2**, 1–70.

Angevine, J. B., Jr 1970 Time of neuron origin in the diencephalon of the mouse. An autoradiographic study. *J. Comp. Neurol.* **139**, 129–188.

Angevine, J. B., Jr & Sidman, R. L. 1961 Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature, Lond.* **192**, 766–768.

Barlow, H. B. 1975 Visual experience and cortical development. *Nature, Lond.* **258**, 199–204.

Bennett, M. R. & Pettigrew, A. G. 1974 The formation of synapses in striated muscle during development. *J. Physiol., Lond.* **241**, 515–545.

Berry, M. & Rogers, A. W. 1965 The migration of neuroblasts in the developing cerebral cortex. *J. Anat.* **99**, 691–709.

Brodmann, K. 1905 Beitage zur Histologischen Lokalization der Grosshirnrinde Dritte Mitteilung: Die Rinderfelder der Niederen Affen. *J. Psychol. Neurol. Leipzig* **9**, 177–226.

Clark, W. E. LeGross 1932 A morphological study of the lateral geniculate body. *Br. J. Ophthal.* **16**, 264–284.

Cowan, W. M., Gottlieb, D. I., Hendrickson, A. E., Price, J. L. & Woolsey, T. A. 1972 The autoradiographic demonstration of axonal connections in the CNS. *Brain Res.* **37**, 21–51.

Grafstein, B. 1971 Transneuronal transfer of radioactivity in the central nervous system. *Science, N.Y.* **172**, 177–179.

Graybiel, A. M. & Nauta, W. J. H. 1976 Quoted in Graybiel, A. M. 1975 Anatomical organization of retinotectal efferents in the cat: An autoradiographic study. *Brain Res.* **96**, 1–23.

Hicks, S. P. & D'Amato, C. J. 1968 Cell migrations to the isocortex in the rat. *Anat. Rec.* **160**, 619–634.

Hubel, D. H., LeVay, S. & Wiesel, T. N. 1975 Mode of termination of retinotectal fibres in macaque monkey: An autoradiographic study. *Brain Res.* **96**, 25–40.

Hubel, D. H. & Wiesel, T. N. 1968 Receptive fields and functional architecture of monkey striate cortex. *J. Physiol., Lond.* **195**, 215–243.

Hubel, D. H. & Wiesel, T. N. 1972 Laminar and columnar distribution of geniculocortical fibers in the macaque monkey. *J. Comp. Neurol.* **146**, 421–450.

Jakob, A. 1928 Das Kleinhirn. In *Handbuch der Mikroskopischen Anatomie des Menschen*, vol. 4, (ed. W. von Möllendorff) pp. 803–843 *Das Nervensystem*. Berlin: Springer.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

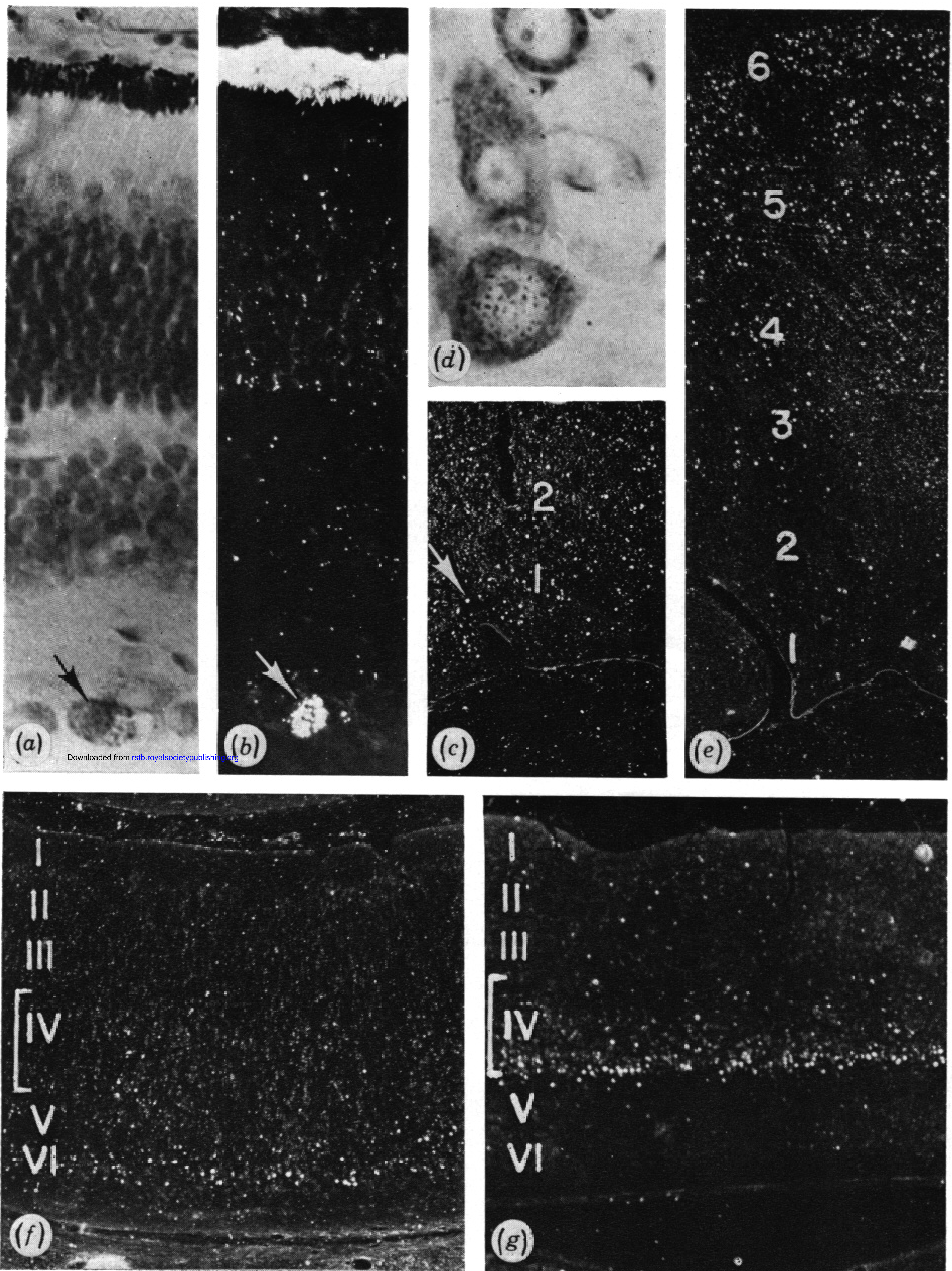
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Jung, R. (ed.) 1973 *Visual centers in the brain. Handbook of sensory physiology*, vol. 7.3. Berlin: Springer.
Kostovic, I. & Molliver, M. E. 1974 A new interpretation of the laminar development of cerebral cortex: synaptogenesis in different layers of neopaliam in the human fetus. *Anat. Rec.* **178**, 395 (abstract).
Larramendi, L. M. H. 1969 Analysis of synaptogenesis in the cerebellum of the mouse. In *Neurobiology of cerebellar evolution and development* (ed. R. Llinás), pp. 803–843. Chicago: Amer. Med. Assoc. Educ. and Res. Fdn.
Leblond, C. P. 1964 Classification of cell populations on the basis of their proliferative behavior. *Natl. Cancer Inst. Monograph* **14**, 119–150.
Lund, R. D. & Bunt, A. H. 1976 Prenatal development of central optic pathways in albino rats. *J. Comp. Neurol.* **165**, 247–264.
Matthews, N. R., Cowan, W. M. & Powell, T. P. S. 1960 Transneuronal degeneration in the lateral geniculate nucleus of the macaque monkey. *J. Anat.* **94**, 145–169.
Minkowski, M. 1920 Ueber den Verlauf, die Endigung und die zentrale Repräsentation von gekreuzten und ungekreuzten Sehnervenfasern bie eingen Säugetieren und biem Menschen. *Schweiz. Arch. f. Neur. u. Psychiat.* **6**, 201–252; **7**, 268–302.
Nowakowski, R. S. & Rakic, P. 1974 Clearance rate of exogenous ^{3}H-thymidine from the plasma of pregnant rhesus monkeys. *Cell Tissue Kinet.* **7**, 189–194.
Polyak, S. 1957 *The vertebrate visual system.* (1390 pages) University of Chicago Press.
Preobrazhenskaya, N. S. 1965 Occipital area, lateral geniculate body, pulvinar and other subcortical structures of the visual analysor. (In Russian.) In *Development of child's brain* (ed. S. A. Sarkisov), pp. 87–127. Leningrad: Medicina.
Price, J. L. & Schowb, J. E. 1976 Initial overlap during development of complementary laminar projections to the piriform cortex. Paper presented at 30th Annual Meeting of the Cajal Club, Louisville, Kentucky.
Rakic, P. 1972 Mode of cell migration to the superficial layers of fetal monkey neocortex. *J. Comp. Neurol.* **145**, 61–84.
Rakic, P. 1973 Kinetics of proliferation and latency between final division and onset of differentiation of cerebellar stellate and basket neurons. *J. Comp. Neurol.* **147**, 523–546.
Rakic, P. 1974*a* Neurons in rhesus monkey visual cortex: Systematic relation between time of origin and eventual disposition. *Science, N.Y.* **183**, 425–427.
Rakic, P. 1974*b* Embryonic development of the pulvinar-LP complex in man. In *Pulvinar-LP complex* (eds I. S. Cooper, M. Riklan & P. Rakic) pp. 3–25. Springfield: Charles C. Thomas.
Rakic, P. 1975*a* Timing of major ontogenetic events in the visual cortex of the rhesus monkey. In *Brain mechanisms in mental retardation* (eds N. A. Buchwald & M. Brazier) pp. 3–40. New York: Academic Press.
Rakic, P. 1975*b* Cell migration and neuronal ectopias in the brain. In *Morphogenesis and malformation of face and brain. Birth defects: original series* (ed. D. Bergsma) vol. 9, pp. 95–129. New York: Liss.
Rakic, P. 1976*a* Differences in the time of origin and in eventual distribution of neurons in areas 17 and 18 of the visual cortex in rhesus monkey. *Expl Brain Res.* Suppl. (in the press).
Rakic, P. 1976*b* Time of origin, mode of migration and pattern of distribution of neurons in the dorsal lateral geniculate body of the rhesus monkey: H^3-thymidine autoradiographic analysis. *Anat. Rec.* **184**, 507–508 (abstract).
Rakic, P. 1976*c* Synaptic specificity in the cerebellar cortex: Study of anomalous circuits induced by single gene mutation in mice. In: *The synapse. Cold Spring Harbor Symp. Quant. Biol.* **40**, 333–346.
Rakic, P. 1976*d* Prenatal genesis of connections subserving ocular dominance in the rhesus monkey. *Nature, Lond.* **261**, 467–471.
Rakic, P. 1977 Tritiated thymidine autoradiographic analysis of the time of origin, routs of migration and pattern of distribution of neurons in the dorsal lateral geniculate body of the rhesus monkey. *J. Comp. Neurol.* (Submitted for publication).
Rakic, P. & Sidman, R. L. 1970 Histogenesis of cortical layers in human cerebellum, particularly the lamina dissecans. *J. Comp. Neurol.* **139**, 473–500.
Shimada, M. & Langman, J. 1970 Cell proliferation, migration and differentiation in the cerebral cortex of the golden hamster. *J. Comp. Neurol.* **139**, 227–244.
Sidman, R. L. 1970 Autoradiographic methods and principles for study of the nervous system with thymidine-H^3. In *Contemporary research methods in neuroanatomy* (eds W. J. H. Nauta & S. O. E. Ebbesson) pp. 252–274. Berlin: Springer.
Sidman, R. L. & Rakic, P. 1973 Neuronal migration, with special reference to developing human brain: a review. *Brain Res.* **62**, 1–35.
Wiesel, T. N. & Hubel, D. H. 1963 Single-cell responses in striate cortex of kittens deprived of vision in one eye. *J. Neurophysiol.* **26**, 1003–1017.
Wiesel, T. N., Hubel, D. H. & Lam, D. M. K. 1974 Autoradiographic demonstration of ocular-dominance columns in the monkey striate cortex by means of transneuronal transport. *Brain Res.* **79**, 273–279.
Wise, S. P. & Jones, E. G. 1976 The organization and postnatal development of the commissural projection of the rat somatic sensory cortex. *J. Comp. Neurol.* **168**, 313–344.
Zecevic, N. & Rakic, P. 1976 Differentiation of Purkinje cells and their relationship to other components of developing cerebellar cortex in man. *J. Comp. Neurol.* **167**, 27–48.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

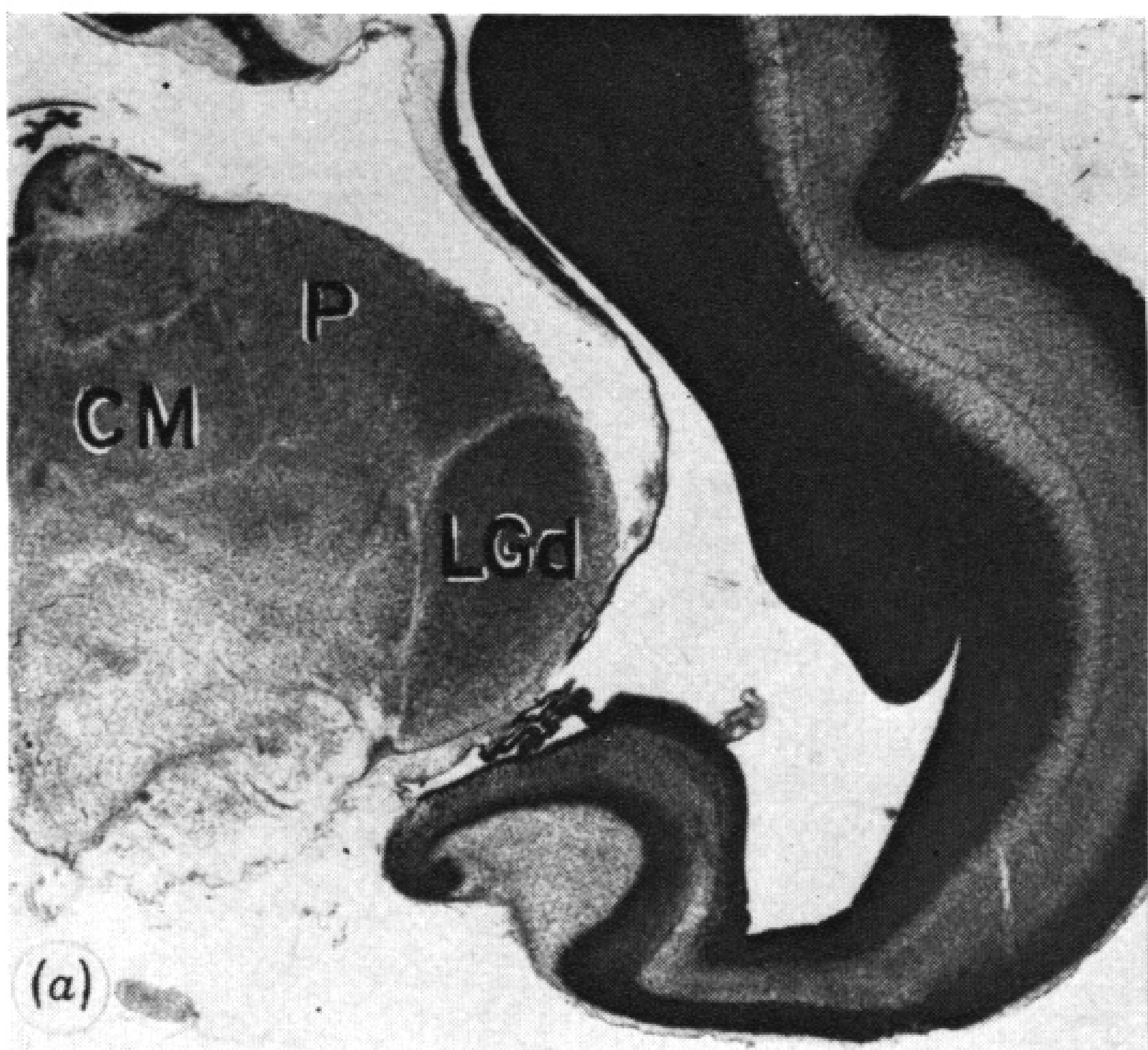


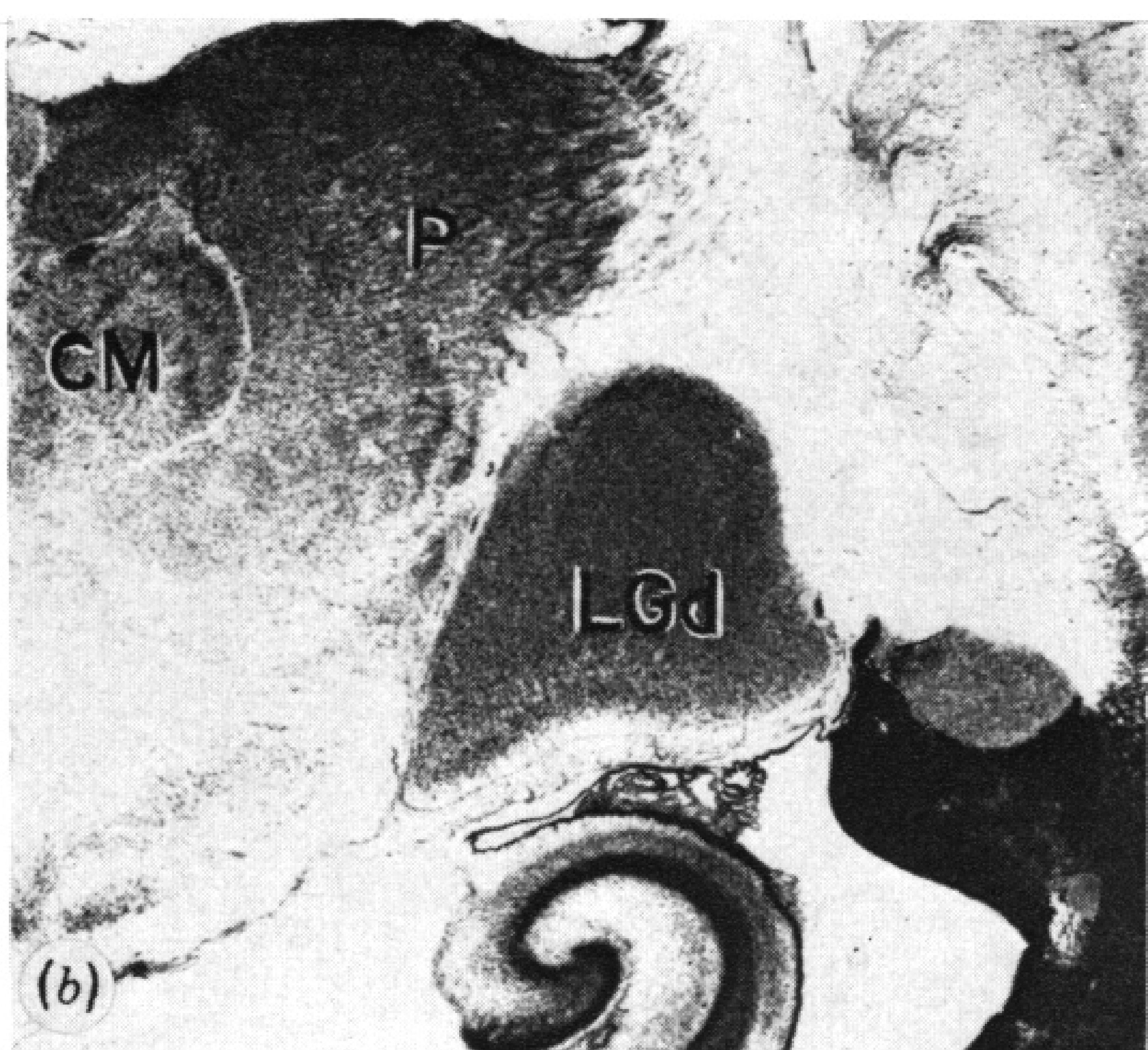
Downloaded from rstb.royalsocietypublishing.org

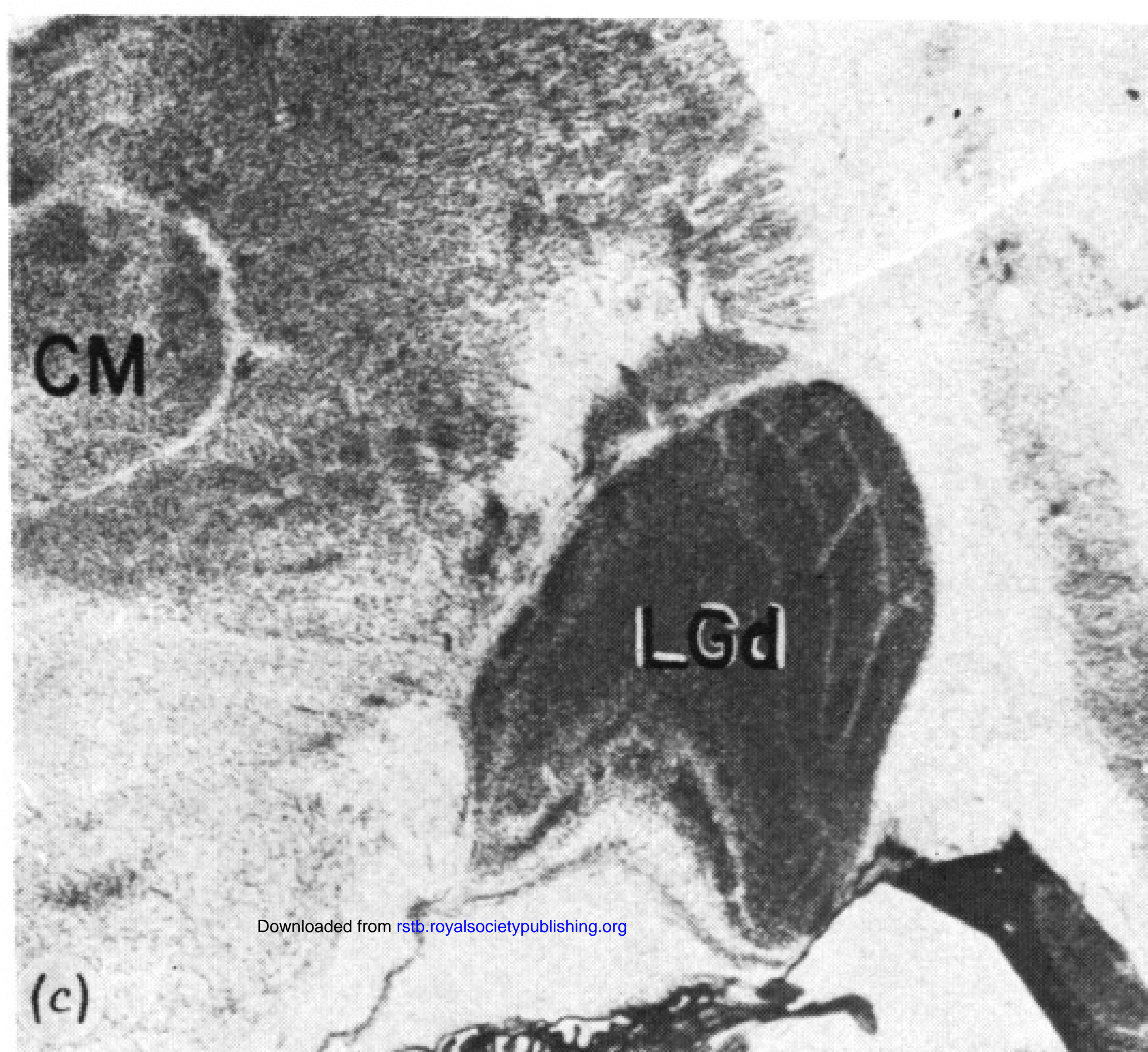
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

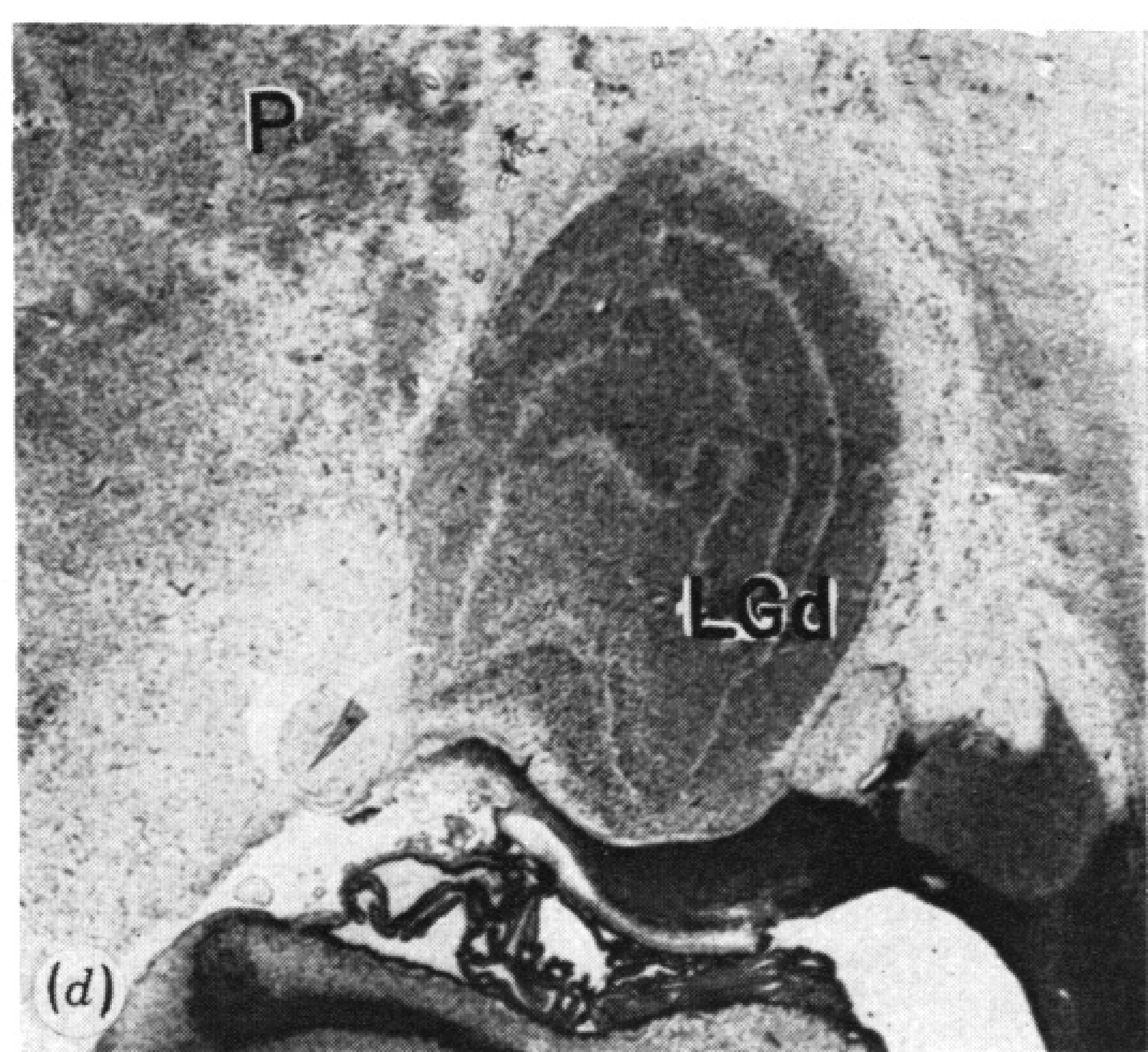
FIGURE 2. Bright- and dark-field photographs of labelled neurons in the visual system of two- to three-month old postnatal (P) monkeys that had been exposed to $[H^3]$thymidine at various embryonic (E) days. (a) Heavily labelled retinal ganglion cell (arrow) in E36–P54 animal. Bright-field photograph across the full thickness of the retina. (b) Similar cell photographed in dark-field illumination. (*c*) Low magnification dark-field photograph of the E38–P60 animal displaying labelled cells in magnocellular layers 1 and 2 of the LGd. (d) Bright-field photograph of the large labelled neuron marked in figure 2*c* by arrow. (e) Low power dark-field photograph across entire thickness of the lateral geniculate body in E43–P70 animal. Labelled neurons are predominantly located in layers 5 and 6. (*f*) Area 17 in the E62–P50 animal. Heavily labelled neurons are located in layer VI and deep zone of layer V. (*g*) Corresponding sector of area 17 in E70–P98 animal. Heavily labelled neurons are concentrated mostly in the deep zone of layer IVC.

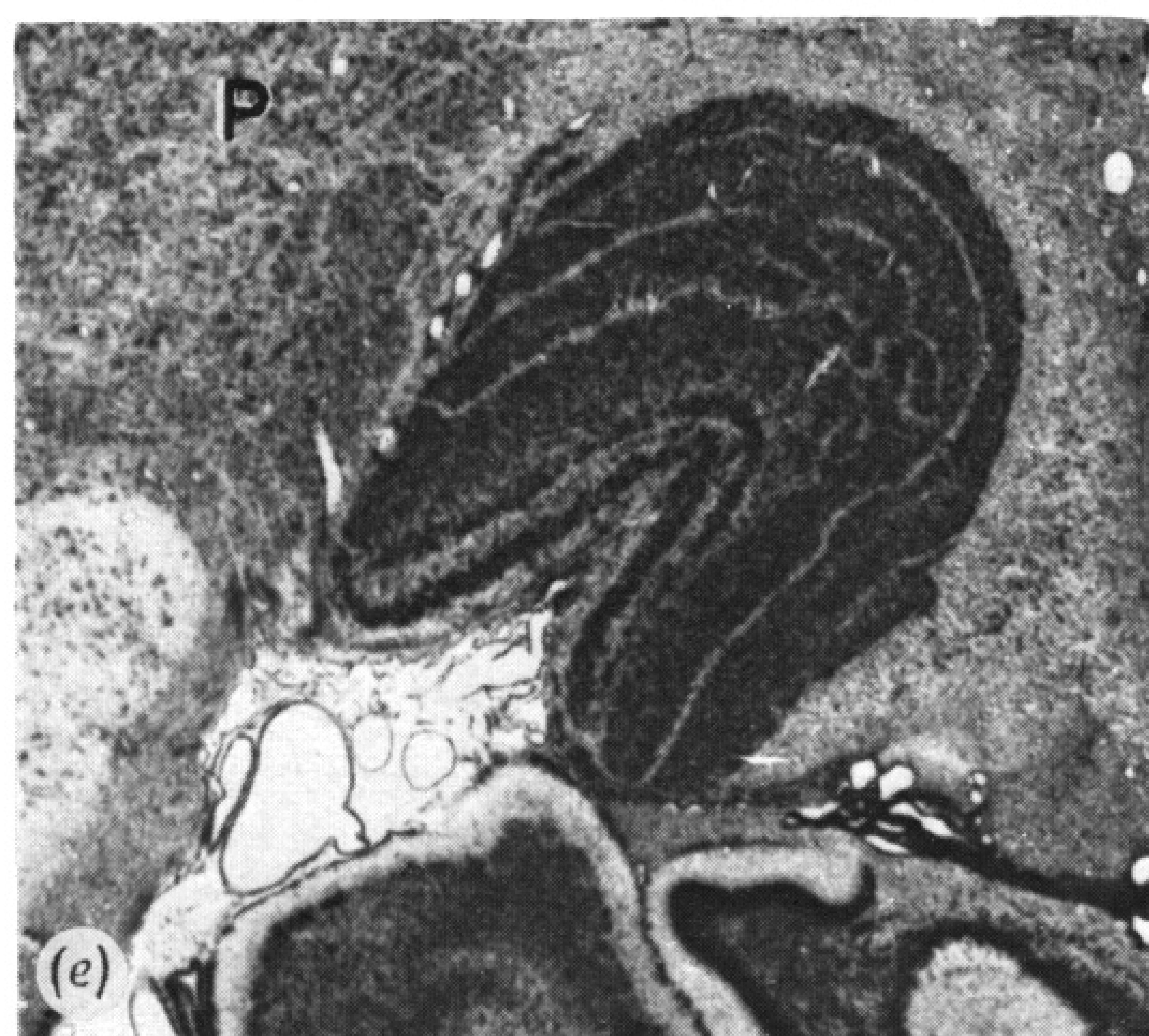


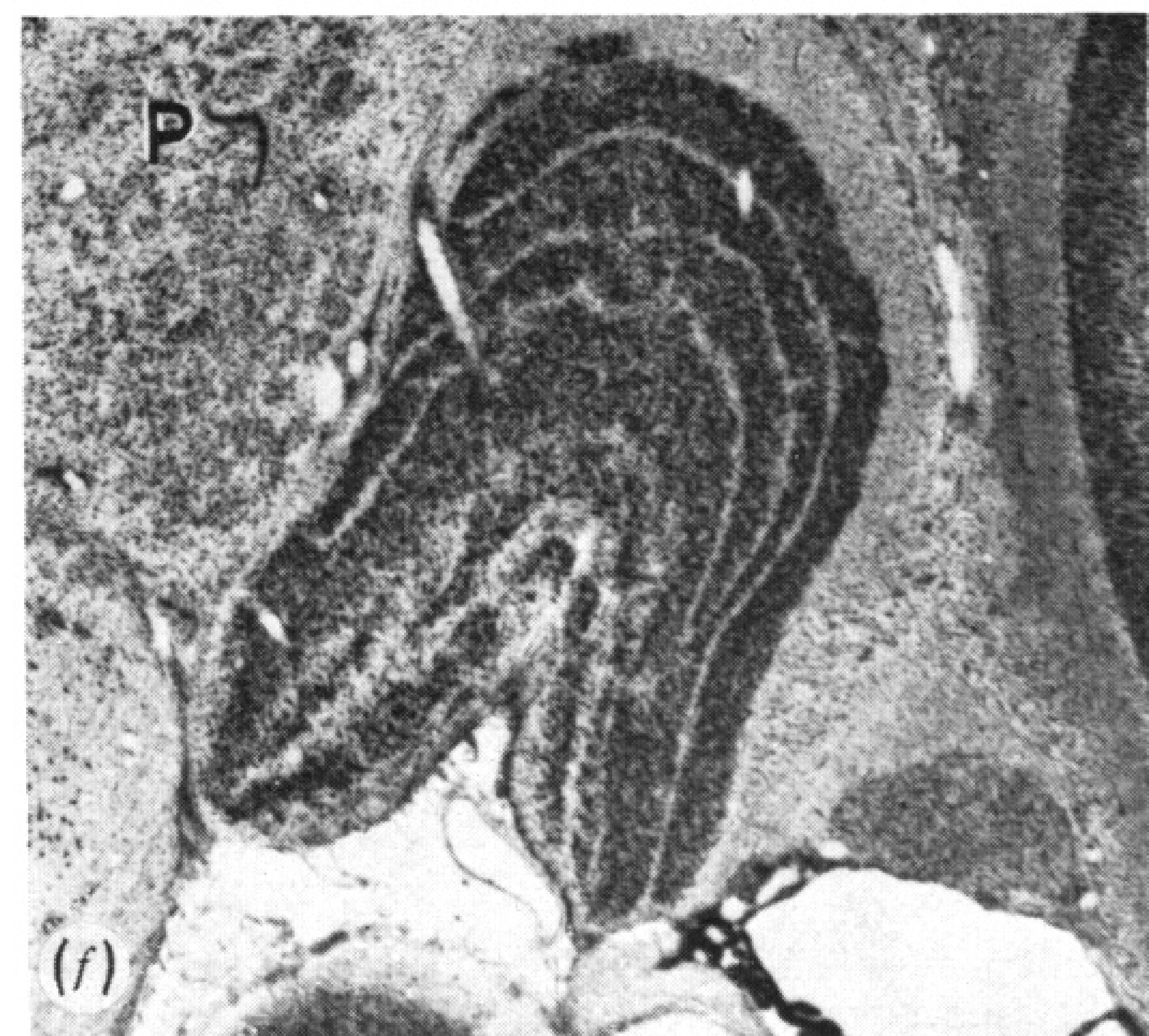




Downloaded from rstb.royalsocietypublishing.org





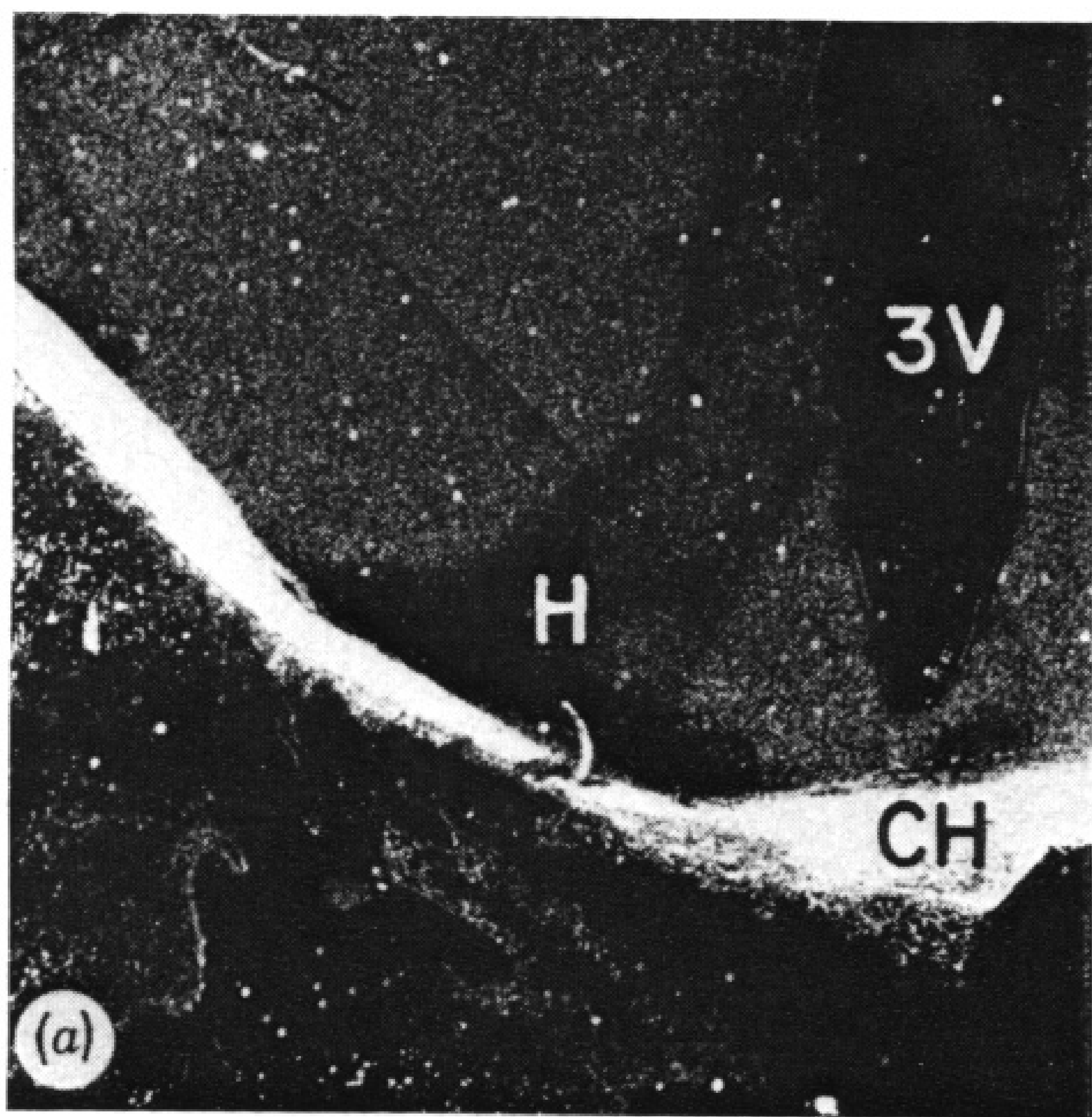


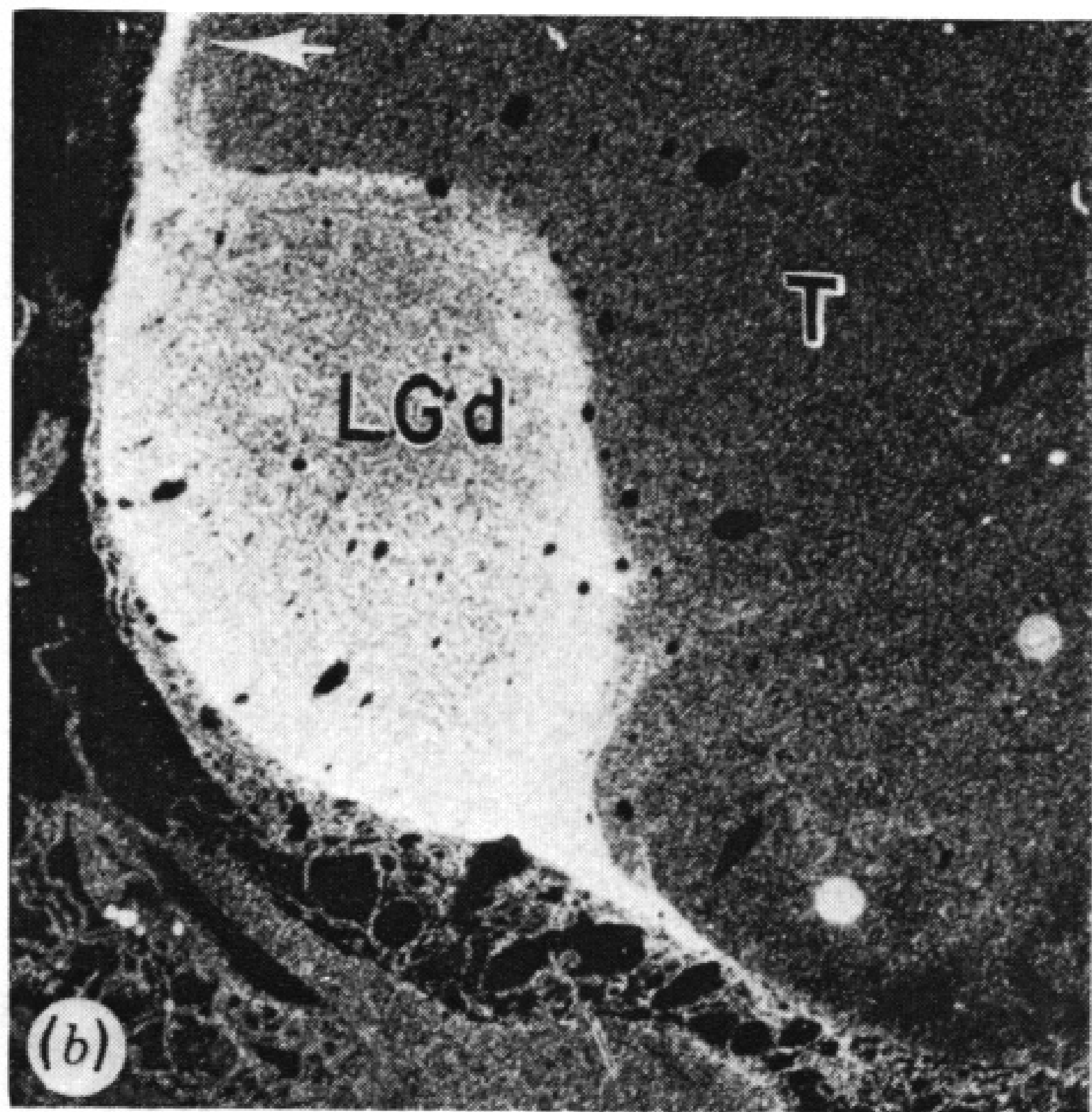
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

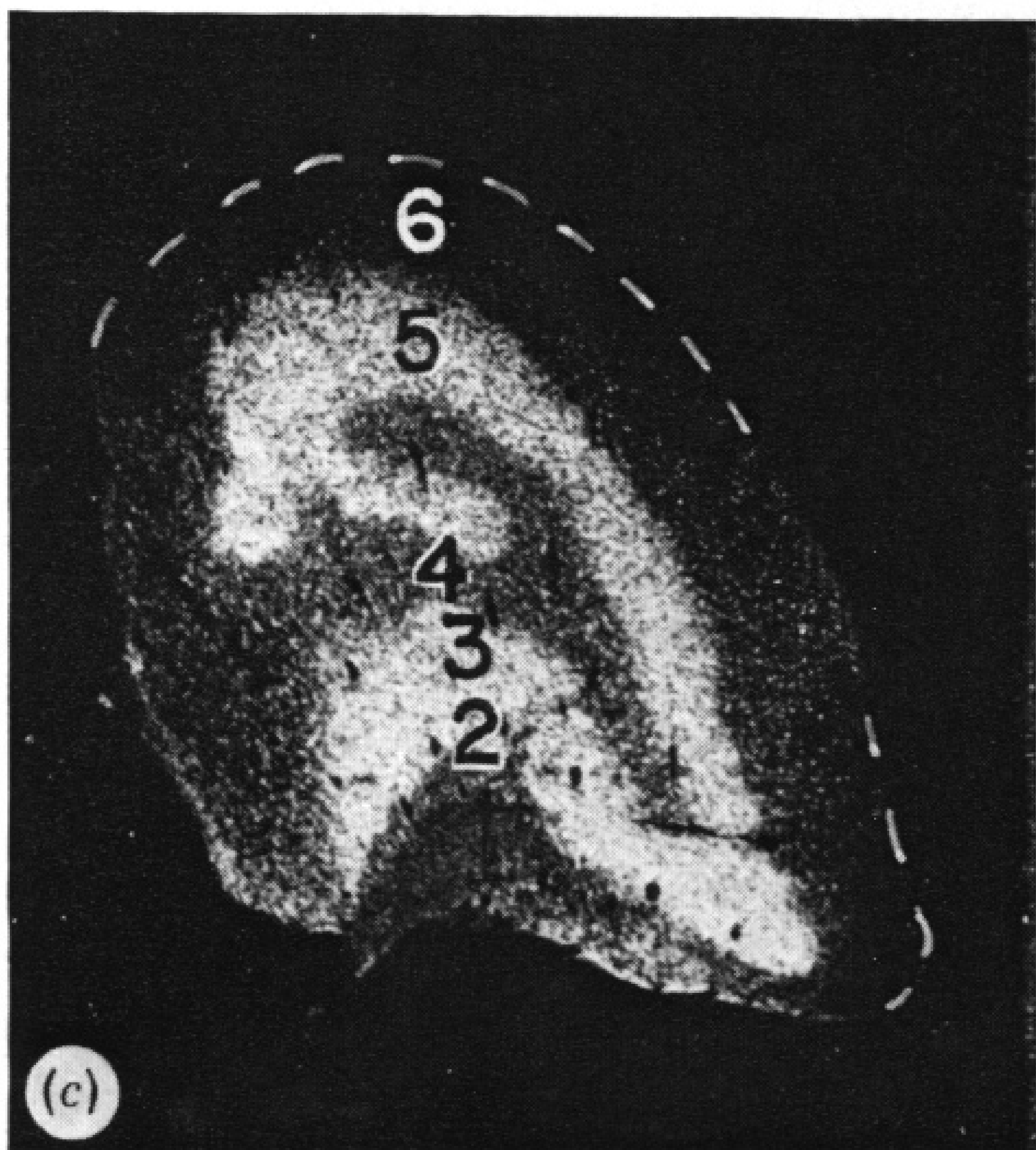
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

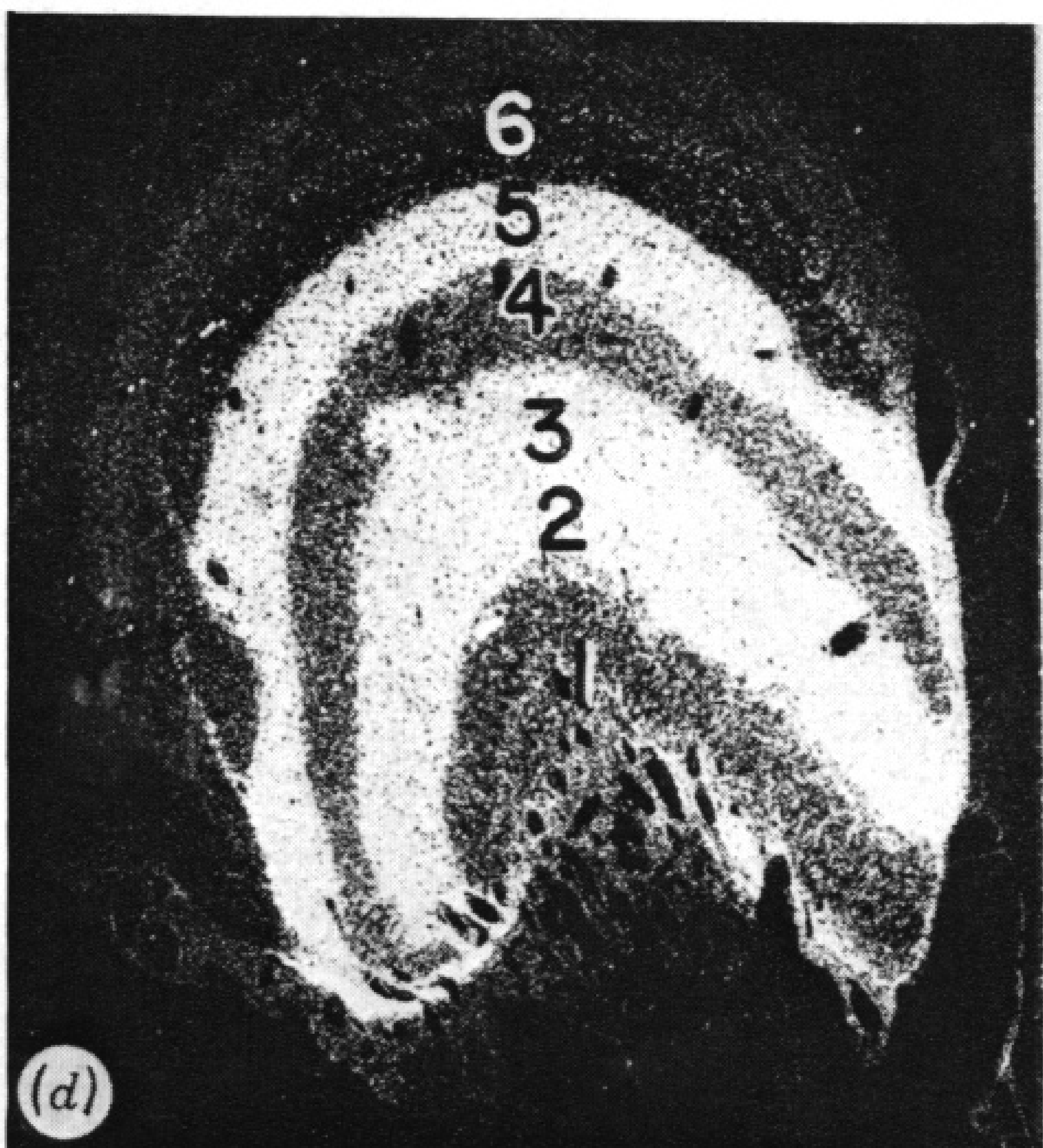
FIGURE 4. The position, shape and cell distribution of the dorsal lateral geniculate body in rhesus monkey diencephalon at different foetal and postnatal ages. Cresyl violet stained 35 μm thick sections, courtesy of Dr P. I. Yakovlev. The foetal ages were estimated by comparing these specimens with specimens cut at 8 μm in my collection of over 80 monkey foetuses in which pregnancies were precisely timed. Estimated age is approximately E70 (at *a*); E90 (at *b*); E110 (at *c*); E125 (at *d*). At *e* is a newborn and at *f* a 91-day-old monkey. Cell free, fibre layers that separate laminae are clearly indicated in older specimens, but are not present at E70 and E90, although numerous axons from both eyes have invaded LGd (see figure 5*b*). Further explanation in text. Abbreviations: CM, nucleus centre medanium; LGd, dorsal lateral geniculate body; P, pulvinar.

5



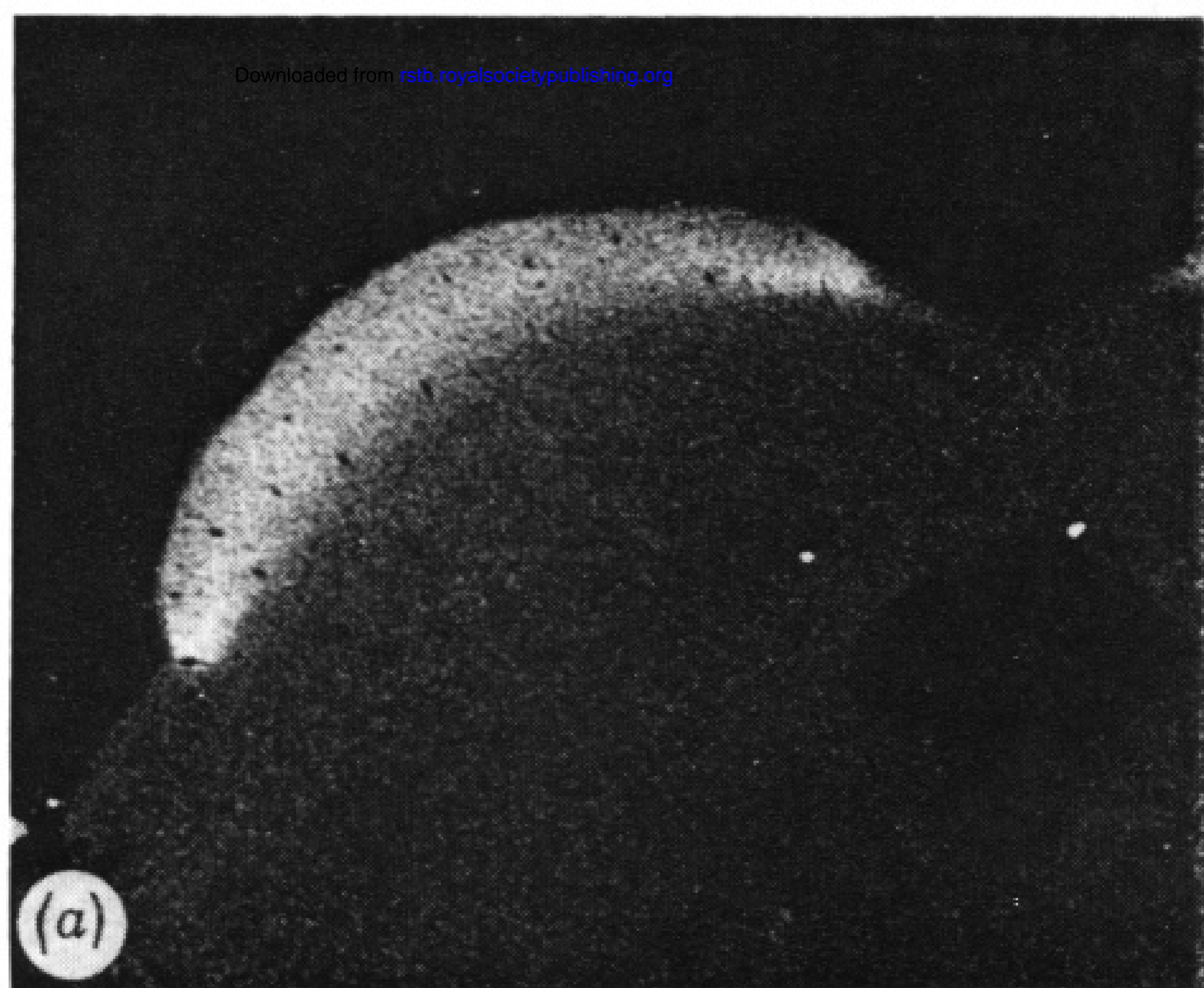


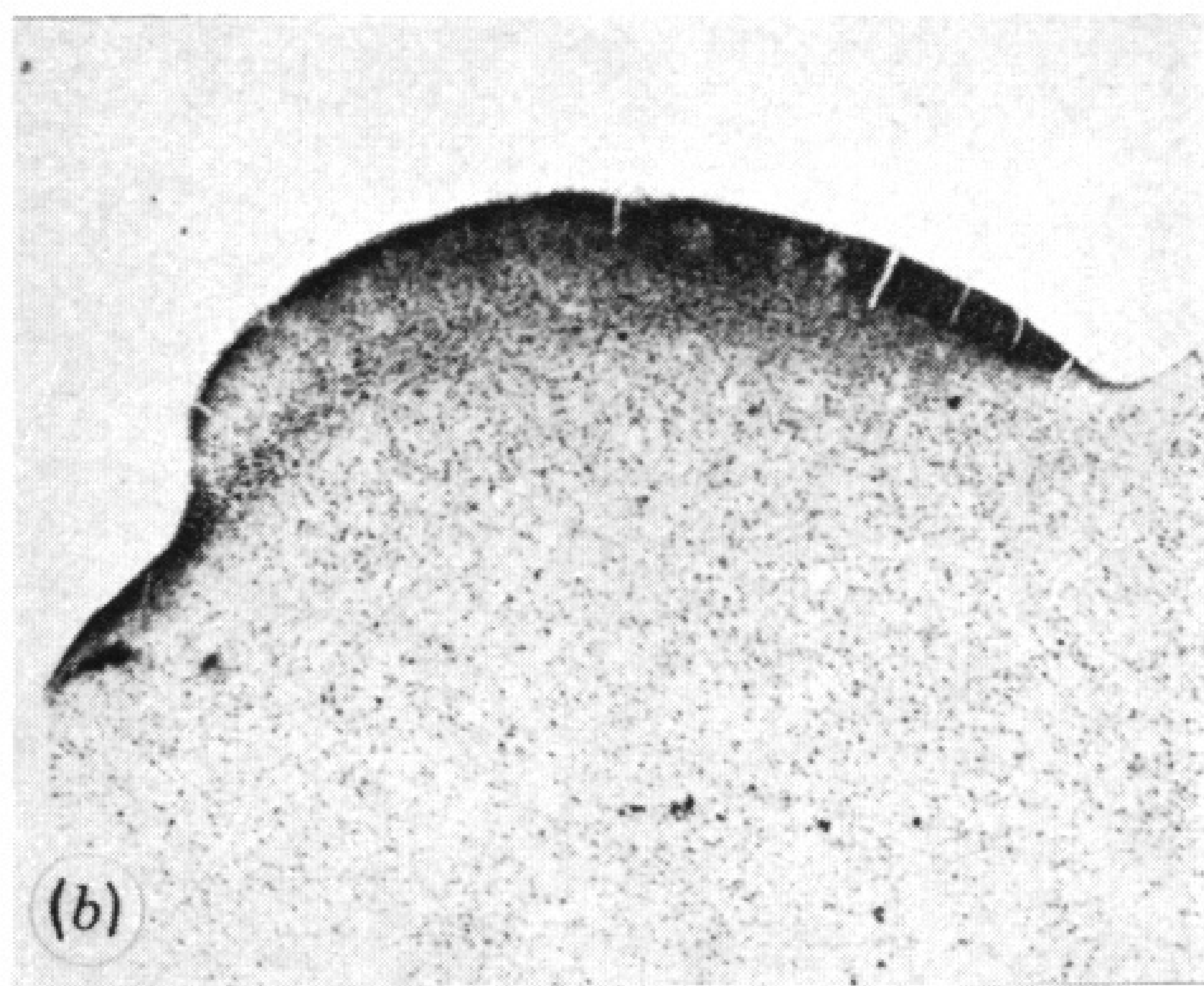


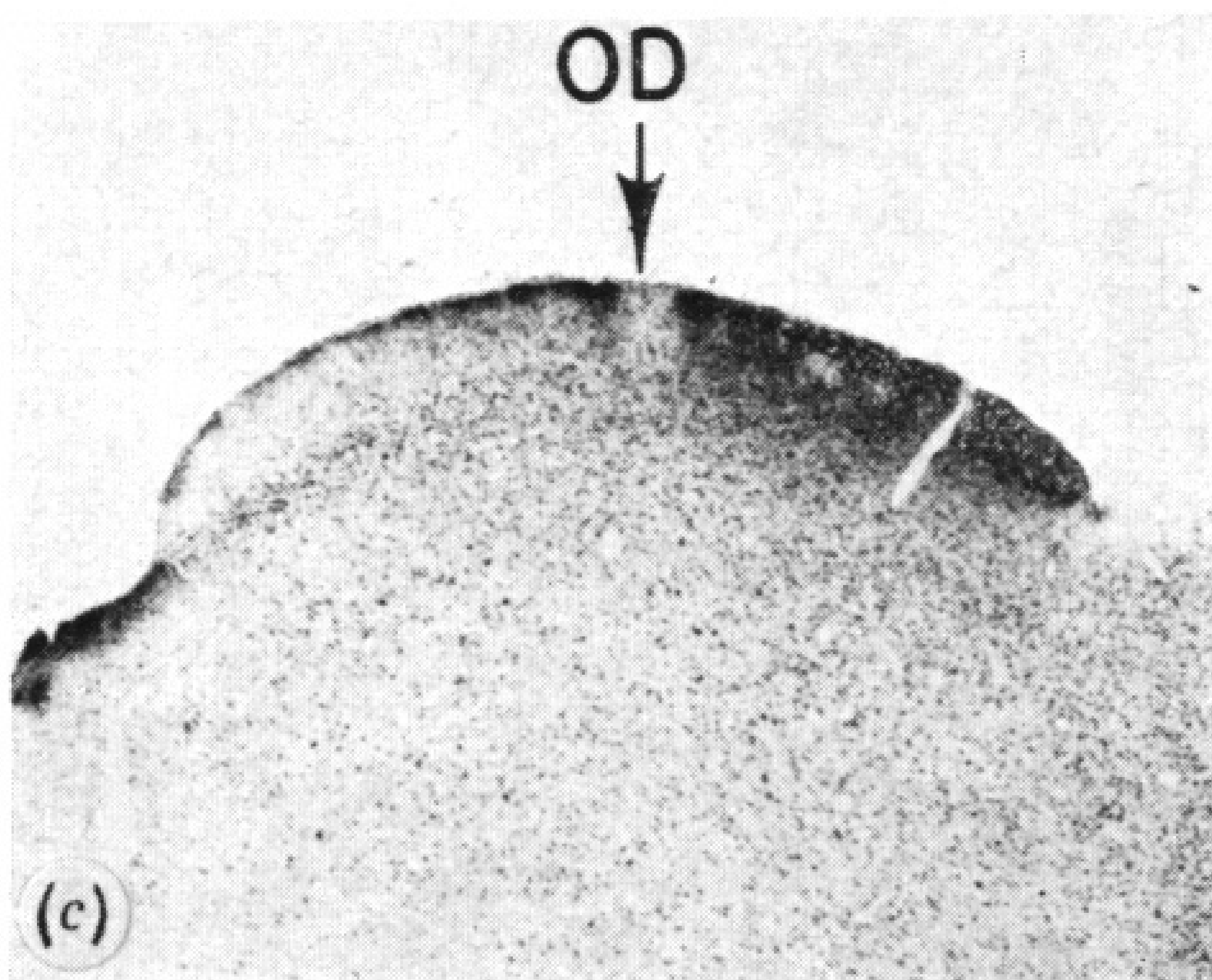


6

Downloaded from rstb.royalsocietypublishing.org







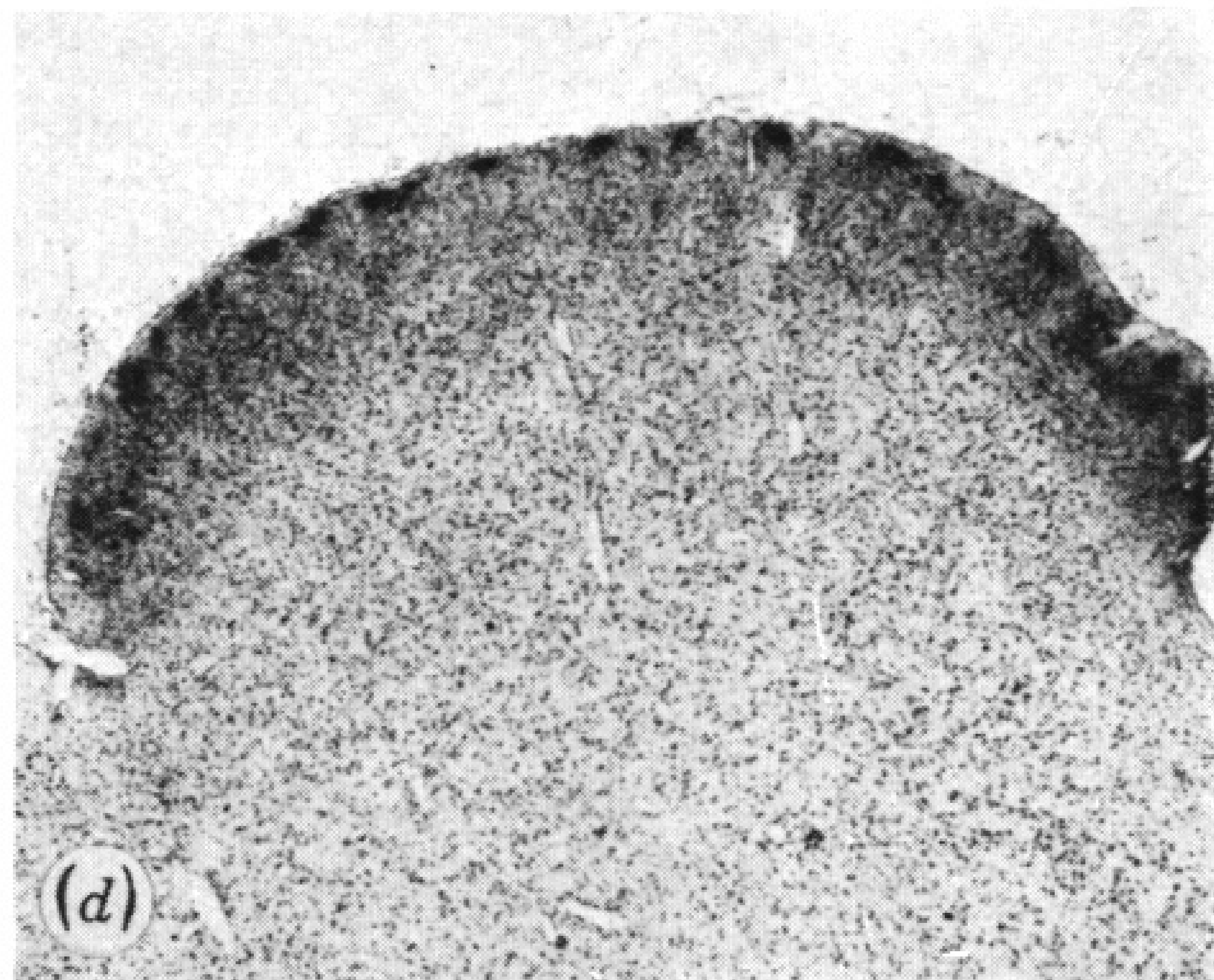
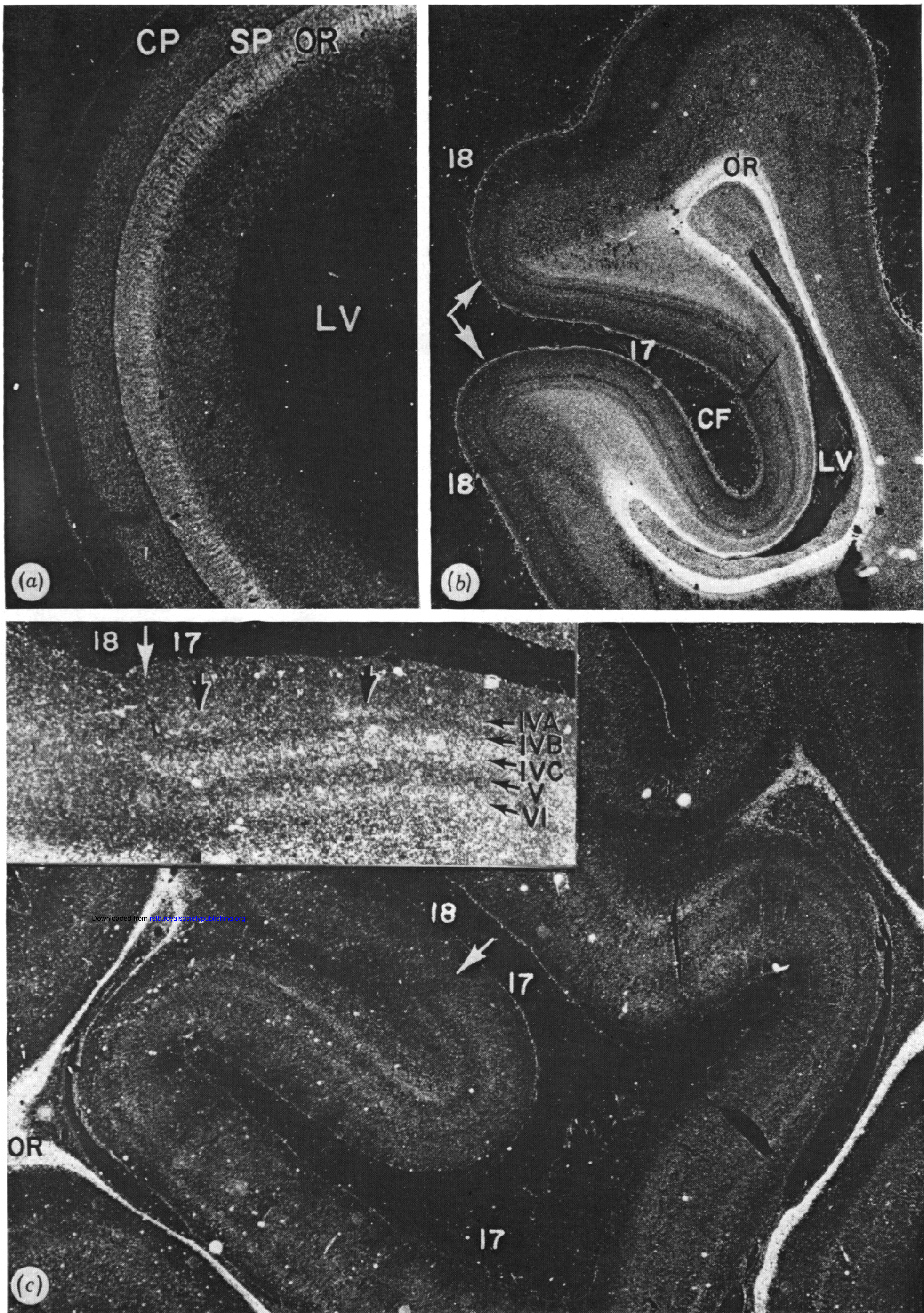


FIGURE 5 AND 6. For description see opposite.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURE 7. (*a*) Dark-field photograph of the occipital lobe of the fetus injected with a mixture of [^{3}H]proline and [^{3}H]fucose at E64, sacrificed at E78. The large lateral ventricle (LV) is on the right. Optic radiation (OR) is heavily labelled by transneuronal transport of isotopes, but fibres stop at the subplate layer (SP) and do not invade the developing cortical plate (CP). (*b*) Occipital lobe of the foetus whose one eye was injected with the same mixture of labels at E110 and sacrificed at E124. The optic radiation surrounds the lateral ventricle (LV) and emanates axons only to area 17 of the calcarine fissure (CF). Projections to the cortex stop sharply at the borderline of area 18 (arrows). Some axons and/or terminals have entered the cortical plate, but are distributed uniformly over layer IV. (*c*) Visual cortex of the foetus injected and sacrificed at a slightly older age (E130–E144). More axons and/or terminals have invaded the cortex and are concentrated over layer V and VI. Segregation of axons and/or terminals over sublayers IVA and IVC, as well as alternating stripes of higher (black arrowheads) and lower grain counts are barely indicated in the dark-field inset of an autoradiogram exposed 16 weeks. For further details, see text and Rakic (1976*d*).